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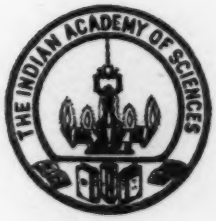

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THE RELATION BETWEEN THE EXCITATORY AND THE CONTRACTILE MECHANISMS OF MUSCLE

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Muscle responds by contraction or relaxation when it is stimulated, the response being produced through the mediation of the excitatory system. The type of response will also be conditioned by the properties of the contractile system. Though the excitatory system may be responsive, the contractile system may not be "excitable"; the contractile system may be unable to contract or relax, though the excitatory system may be functioning "normally". To know the behaviour of the contractile system in a response, it is therefore necessary to exclude the excitatory system.

Szent-Gyorgyi has introduced the glycerinised muscle to study the contractile mechanism. In another method, substances are injected directly into the fibres of striated muscle (Heilbrunn, 1952). By the latter method, it has been found that the contractile system of striated muscle is very sensitive to calcium (Heilbrunn and Wiercinki, 1952); barium ions act like calcium ions in shortening of the muscle protoplasm. Magnesium acts very weakly. Adenosine triphosphate also causes contraction. According to Heilbrunn, muscular contraction is akin to coagulation of blood.

The method of injecting substances into the striated muscle fibre to determine the response of the contractile mechanism is not free from objection. It is quite possible that the muscle may be responding *via* the excitatory system and not by direct action of the injected substance on the contractile mechanism. In fact, there is evidence, that the muscle may be excited by substances acting on the muscle membrane from within the fibres (Singh, 1938, 1939, 1942, 1944, 1945). Thus, when the osmotic pressure of the saline is increased suddenly, the muscle may contract. Increase in osmotic pressure of the saline increases the concentration of ions within the fibres, chiefly potassium (Gokhale and Singh, 1945), and this sudden increase in contraction of ions within the fibres acts like increase outside and causes contraction or inhibition. Thus the muscle membrane is sensitive from without as well as from within the cells, though it appears to be less sensitive from the inside.

The contractile mechanism of unstriated muscle has been studied by two methods (Singh and Singh, 1949, 1950, 1951, 1954, *a*, *b*, *c*). In the first method, the muscle is allowed to die in the experimental solution, which

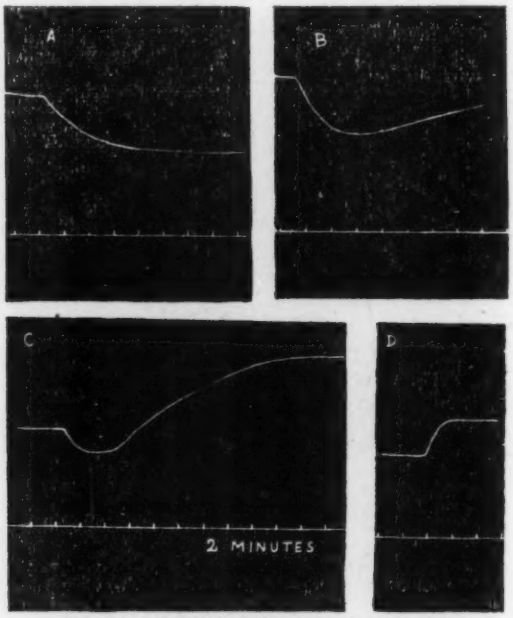

FIG. 1. Heat killed dog's stomach muscle.

A. Effect of 40 per cent. hydrochloric acid. B. Effect of 50 per cent. C. Effect of 70 per cent. D. Effect of 90 per cent.

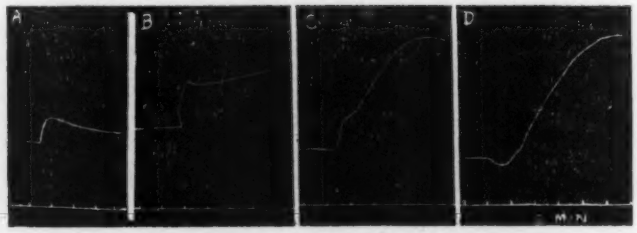

FIG. 2. Heat killed dog's stomach muscle.

A. Effect of 100 per cent. hydrochloric acid. B. Effect of 90 per cent. C. Effect of 80 per cent. D. Effect of 70 per cent.

is not oxygenated. It is well known that the normal permeability of the muscle depends upon metabolism, so that as the muscle dies, it becomes inexcitable as well as fully permeable to the substances; the excitatory

system then ceases to function and the contractile system acts without interference. In the second method, the muscle is killed by heat. Most of the

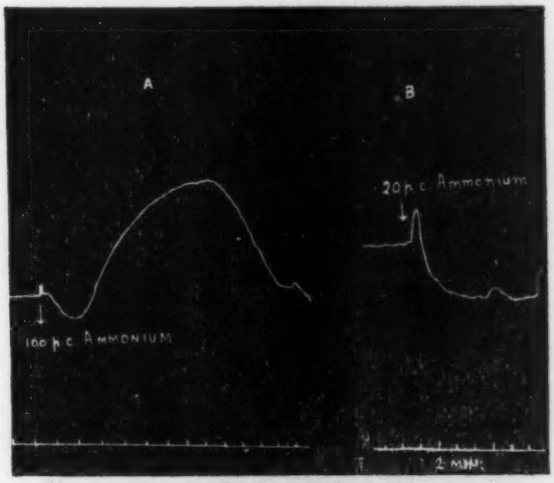

FIG. 3. Dog's stomach muscle.

Effect of ammonium saline. A. Effect of 100 per cent. ammonium. B. Effect of 20 per cent. ammonium.

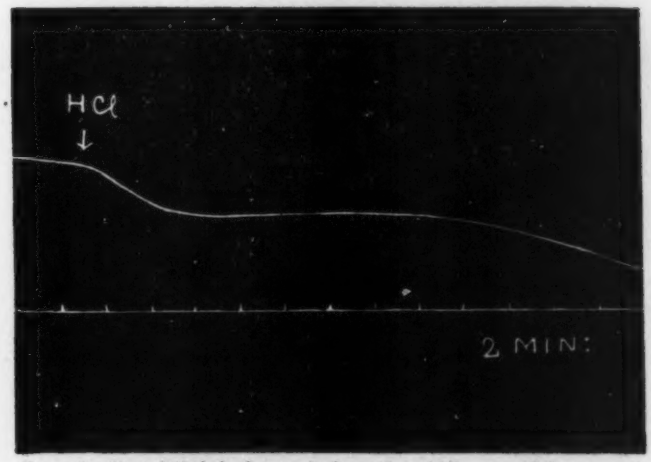

FIG. 4. Heat killed dog's muscle from the middle part of the stomach. Effect of 70 per cent. hydrochloric acid.

results obtained by the former method have been substantiated by the latter method. It has been found that substances which denature proteins cause

active or passive relaxation of unstriated muscle, and protein coagulants cause contraction.

Some of the results obtained by the dying muscle method agree with those obtained in striated muscle by the mioroinjection method. Thus

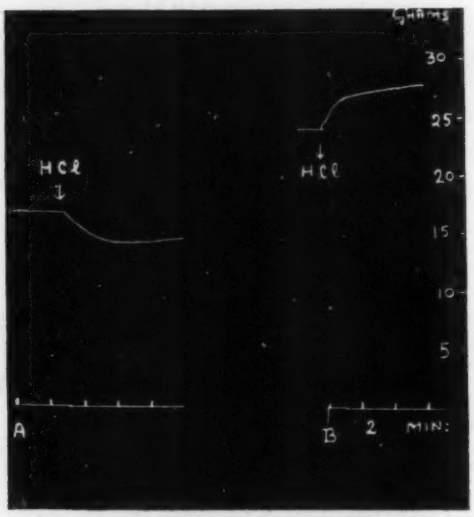

FIG. 5. Heat killed frog's rectus abdominis.

A. Effect of 70 per cent. hydrochloric acid, with initial length 23 mm. B. Same as in A with initial length 27 mm.

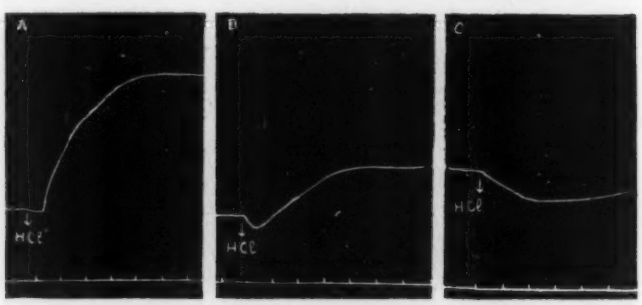

FIG. 6. A. Heat killed dog's stomach muscle. Effect of 70 per cent. hydrochloric acid.

A. From the cardiac end. B. From the middle part. C. From the middle part but near the pylorus.

in *Mytilus* muscle, both calcium and barium cause contraction of the contractile mechanism, as found in striated muscle. In frog's stomach muscle,

barium causes relaxation, so that the contractile mechanism of *Mytilus* muscle, resembles that of striated muscle; a common physiological property of both is the absence of active relaxation.

In the present research, contraction, relaxation and latency relaxation have been studied, the response being produced by direct action on the

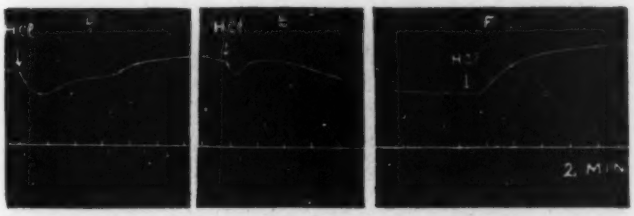

FIG. 6. B. Heat killed dog's stomach muscle. Effect of 70 per cent. hydrochloric acid. D. From the middle but nearer the pylorus than C. E. From nearer the pylorus than D. F. From the pylorus.

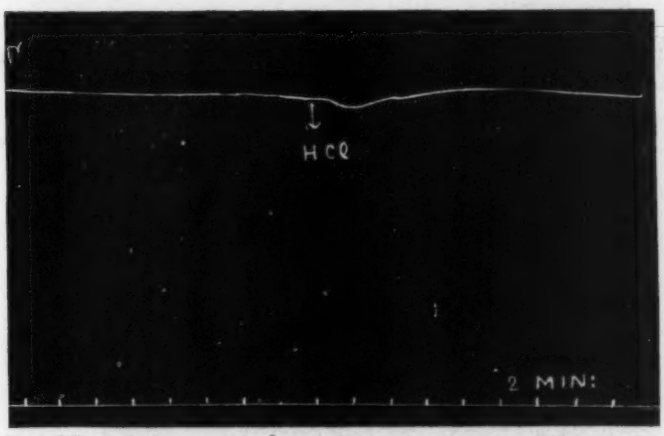

FIG. 7. Heat killed dog's stomach muscle from the pylorus. Effect of 70 per cent. hydrochloric acid.

contractile mechanism; these responses were compared with those produced through the mediation of the excitatory system.

EXPERIMENTAL

These experiments were performed on heat killed muscles (Singh and Singh, 1954, *a*, *b*, *c*). Pieces were obtained from the circular muscle of dog's stomach and stomach of the frog *Rana tigrina*. For experiments on striated muscle, the frog's rectus abdominis was used. For experiments

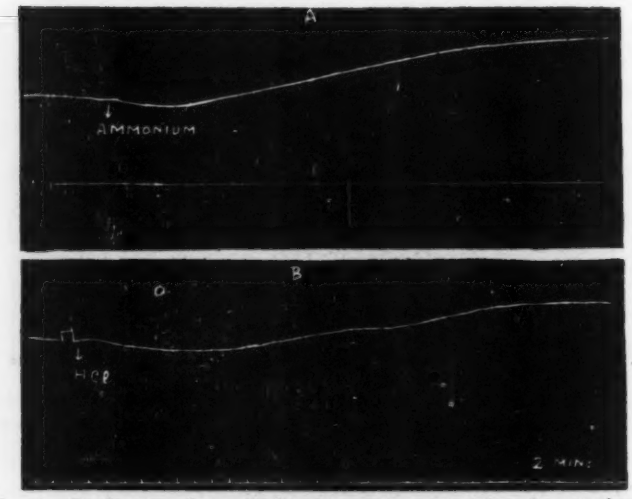

FIG. 8. A. Dog's stomach muscle from the cardiac end. Effect of ammonium. B. Heat killed dog's stomach muscle from the cardiac end. Effect of 70 per cent. hydrochloric acid.

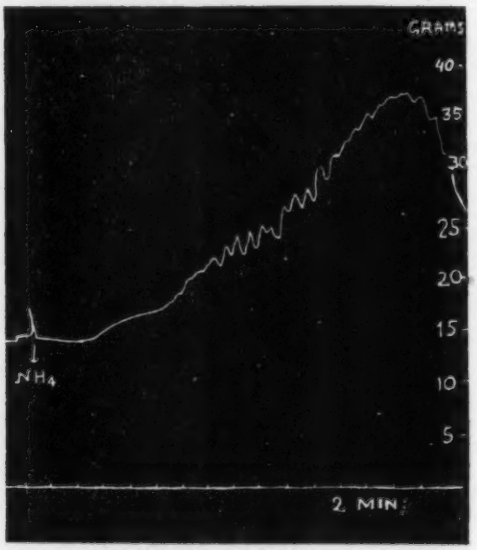

FIG. 9. Dog's stomach muscle from the pyloric end. Effect of ammonium.

on cardiac muscle, pieces from dog's ventricle were employed. From dog's heart, rings of ventricular muscle were removed and cut across at one place,

so as to prepare an oblong piece for experimental use. In such a strip, the muscle bundles would be placed in various directions, but still a good contraction was produced.

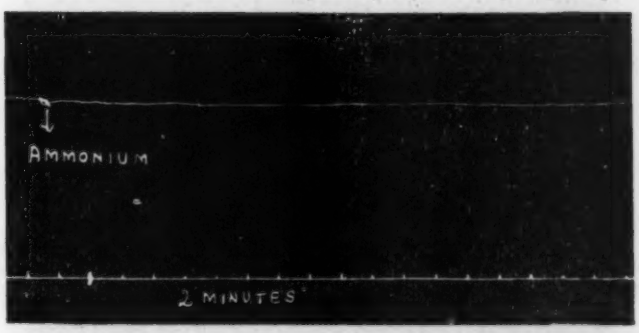

FIG. 10. Dog's stomach muscle from the pyloric end. Effect of ammonium.

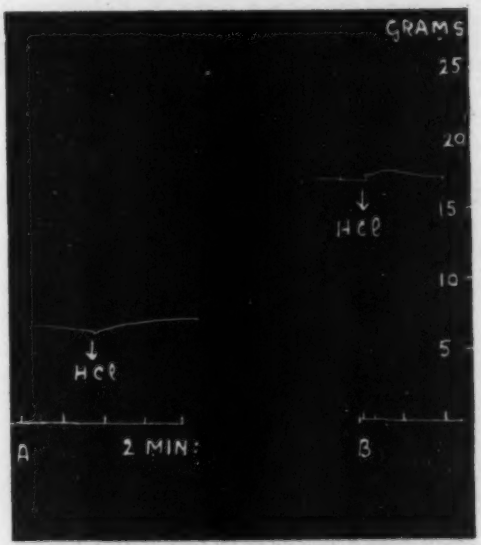

FIG. 11. Heat killed dog's muscle from the middle part of the stomach. Heated to 70° C. for 10 minutes. Effect of 70 per cent. hydrochloric acid.

A. Initial length 33 mm. B. Initial length 38. mm.

Heat killed muscles are contracted by some protein coagulants. A convenient substance formed to cause contraction of loaded muscles was strong hydrochloric acid. Varying volumes of concentrated hydrochloric

acid (A.R., B.D.H.) were dissolved in water, and in this paper the strength of hydrochloric acid used is expressed as the percentage of concentrated hydrochloric acid so dissolved, the strength of the concentrated acid being

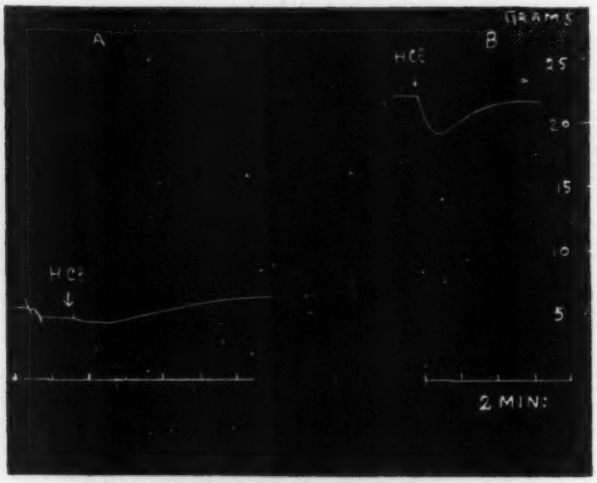

FIG. 12. Heat killed dog's stomach muscle from the middle part of the stomach. Heated to 60° C. for 10 minutes. Effect of 70 per cent. hydrochloric acid.

A. Initial length 32 mm. B. Initial length 37 mm.

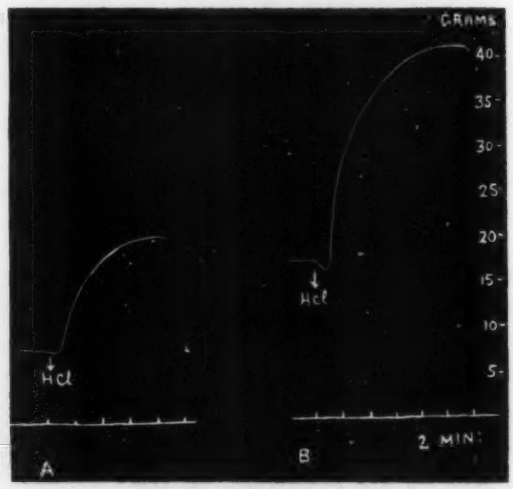

FIG. 13. A. Heat killed dog's stomach muscle from the middle part of the stomach. Effect of 70 per cent. hydrochloric acid.

A. Initial length 33 mm. B. Initial length 38 mm.

considered as 100 per cent. Though strong hydrochloric acid is anything but a physiological stimulus to the contractile mechanism, yet the results

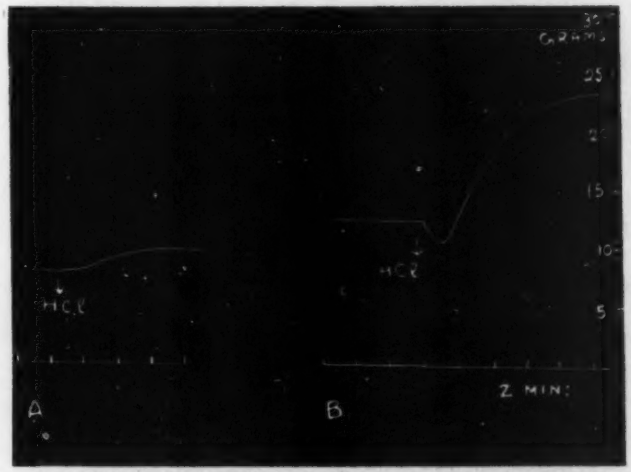

FIG. 13. B. Heat killed dog's stomach muscle from the middle part of the stomach. Effect of 70 per cent. hydrochloric acid.
A. Initial length 30 mm. B. Initial length 34 mm.

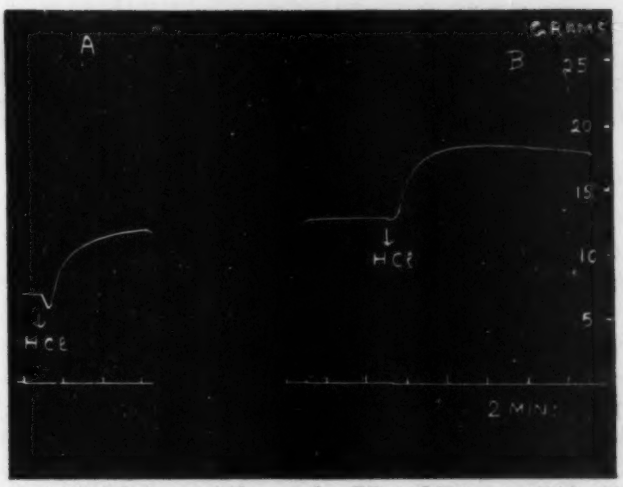

FIG. 14. Heat killed dog's ventricular muscle. Effect of 70 per cent. hydrochloric acid.
A. Initial length 72 mm. B. Initial length 77 mm.

obtained stimulated the physiological responses very closely. To produce the physiological response through the excitatory mechanism, the living

muscle was stimulated with saline, the sodium of which had been replaced with ammonium, as with this substance, the latency relaxation is easily produced in unstriated muscle of dog's stomach (Singh, 1953). The strength of ammonium saline is expressed as percentage of sodium replaced; if all the sodium had been replaced, it is just termed as ammonium saline.

With use of strong hydrochloric acid solutions, a piece of muscle can be used only once. To produce comparable and consistent results, identical pieces of equal length of breadth have to be taken from the same segment of the stomach, as the sensitivity of the contractile mechanism varies in different parts of the organ.

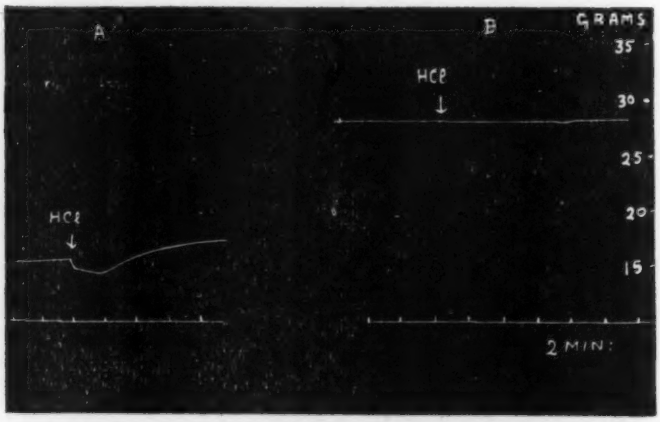

FIG. 15. Heat killed dog's stomach muscle. Effect of 70 per cent. hydrochloric acid.
A. Initial length 27 mm. B. Initial length 31 mm.

The muscles were killed by gradually raising the temperature of the saline from room temperature to 50° C. and keeping at that temperature for 5 minutes, if dog's stomach muscle was used. This minimum period is sufficient to make the muscle inexcitable to strongest electrical or chemical stimuli, such as saturated solutions of potassium or calcium chlorides; it is therefore presumed to be dead. This presumption is supported by the fact, that heating for a longer period, such as 10, 20, 30, 40 minutes does not make significant difference in the results; with such prolonged periods the muscle is certainly dead. The muscle may be heated to 55° C. for similar periods, usually 10 minutes being used in these experiments, without altering the nature of the results. Frog's stomach muscle was heated to 50° C. and kept at that temperature for 10 minutes. Frog's rectus abdominis

and dog's heart muscle were similarly treated. With such treatment, striated muscle enters into a pronounced rigor.

Concentrations of hydrochloric acid used in these experiments were varied in steps of 10 per cent. Most of the experiments were performed on dog's stomach muscle, unless otherwise stated.

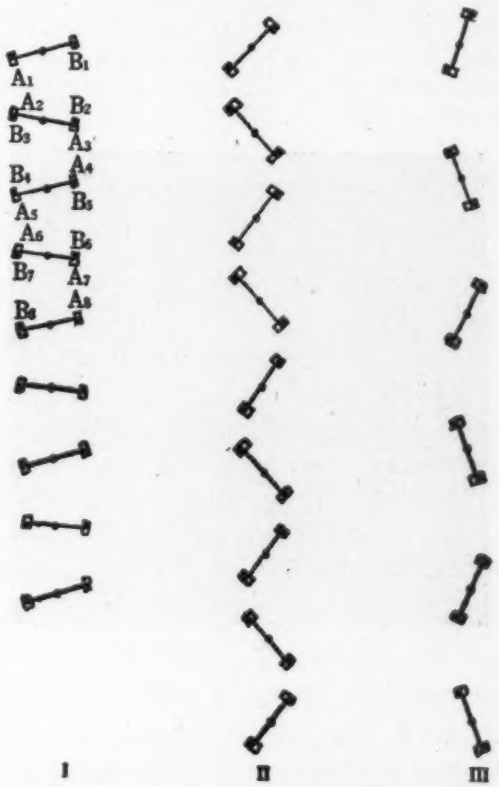

FIG. 16. Diagrammatic representation of the effect of increase in initial length on tension.

I. Representation at a certain length. II. Effect of stretching so that the points of attraction come nearer. III. Effect of further stretching, so that the points of attraction move further apart.

RESULTS

Effect of hydrochloric acid concentration.—Small concentrations of hydrochloric acid, 10 to 40 per cent., produce relaxation only of dog's stomach muscle. Maximum tension is produced by 70 per cent.; as the concentration

of hydrochloric acid is increased, the relaxation is succeeded by contraction, so that with high concentrations, it becomes less and less and ultimately disappears with 80 to 100 per cent. hydrochloric acid (Figs. 1, 2).

Ammonium produces similar results on living muscle. Small concentrations, 10 to 50 per cent. produce relaxation only; with higher concentrations, relaxation becomes less and less, so that it may ultimately disappear altogether (Fig. 3). This is a well-known result in unstriated muscle. Substances which cause contraction, produce relaxation in small concentrations. In *Mytilus* muscle, small voltages of alternating current produce relaxation, whilst larger voltages produce contraction. Similarly, small concentrations

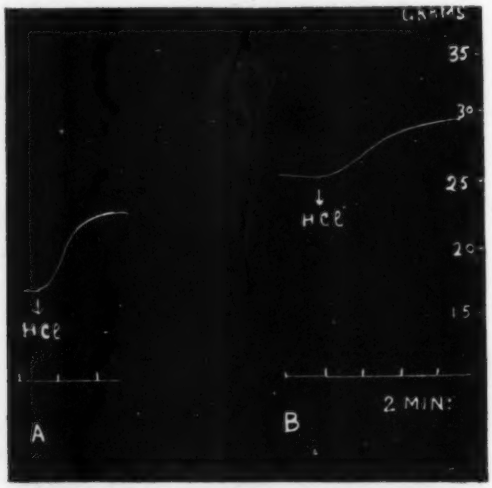

FIG. 17. Heat killed dog's stomach muscle. Effect of 10 per cent. hydrochloric acid.
A. Initial length 32 mm. B. Initial length 37 mm.

of ammonium, potassium, calcium, strontium, bromide, nitrate, iodide, thiocyanate, adrenaline, acetylcholine, caffeine, veratrine, cause relaxation, whilst larger concentrations cause contraction. It is known that small concentrations of adrenaline may cause vasodilatation, whilst larger doses cause vasoconstriction. Similarly, weak stimuli applied to nerves may produce dilator effects, and strong stimuli, constrictor effects.

Effect of sensitivity of muscle.—The sensitivity of dog's stomach muscle is variable. If the muscle from the middle part of the stomach does not contract, then it only relaxes (Fig. 4). Ammonium produces similar results on living muscle. This is also a well-known result on unstriated muscle.

Thus in *Mytilus* muscle small voltages of alternating current cause relaxation; if the excitability of the muscle is decreased by excess of lithium, ammonium, potassium, bromide, nitrate, iodide, thiocyanate, diminution of the calcium concentration of the saline, by excess of adrenaline, acetylcholine, veratrine, caffeine, strontium, barium, then stronger stimuli cause relaxation. Fatigue, as it diminishes excitability, may also convert a vasoconstrictor effect into a vasodilator one; thus whilst the first injection of pituitrin produces rise of blood pressure, a second dose soon after produces a fall of blood pressure.

Effect of tonus.—Dog's stomach muscle, if it shows much contraction on heating, is relaxed by hydrochloric acid. Similarly frog's rectus abdominis goes into rigor when heated; hydrochloric acid causes it to relax. If the muscle is stretched so as to undo the heat contraction, then hydrochloric acid produces contraction (Fig. 5).

Ammonium produces similar effects on living muscle. This is a well-known property of unstriated muscle. Thus when a stimulus, electrical, chemical or nervous, which when applied to the relaxed muscle, causes contraction, will when applied to the tonically contracted muscle, often provokes a rapid relaxation.

Sensitivity of dog's stomach muscle.—Pieces from various parts of the dog's stomach may vary in their response to hydrochloric acid. Those from the cardiac end are most sensitive; further towards the pylorus, the sensitivity decreases (Fig. 6), though very near the pylorus it may again increase. The muscle from near the pylorus does not relax appreciably; it may or may not contract (Fig. 7). Latency relaxation is best obtained from the middle part of pyloric region, and feeble near the cardiac end (Fig. 8).

Ammonium produces identical results on living dog's stomach muscle. The pyloric end does not relax, it may or may not contract (Figs. 9, 10). The latency relaxation is best marked in muscle from middle part of the stomach; it is feeble at the cardiac end and absent at the pylorus (Figs. 8, 9). The experiments with hydrochloric acid and ammonium should be performed on pieces from the same stomach.

The response of the middle and cardiac parts of the stomach muscle to hydrochloric acid can be made to resemble that of the pyloric region, if the former are heated to 70° C. (Fig. 11). The muscle then does not relax, and contraction is feeble. Further heating up to 80 or 90° C. produces similar results. Heating to 60° C. is only partially effective (Fig. 12). Heating up to 55° C. does not change the response.

Effect of initial length on tension.—Increase in initial length up to a certain point increases the tension produced by the action of hydrochloric acid on heated muscles; this has been found with dog's stomach muscle (Fig. 13), frog's rectus abdominis (Fig. 5), dog's heart muscle (Fig. 14). Thus this important property of muscle is a feature of the contractile mechanism.

Effect of initial length on relaxation.—Relaxation of heated dog's stomach muscle by hydrochloric acid increases with increase in initial length up to a certain extent; thereafter it decreases (Fig. 15). Ammonium produces similar effects on living muscle. Increase in initial length may abolish the inhibitory effect of ammonium and other inhibitory substances in living muscle (Singh, 1942, 1953).

Effect of initial length on latency relaxation.—Latency relaxation in heated dog's stomach muscle increases with increase in initial length to a certain extent (Fig. 13), and then decreases. Ammonium produces similar effects in living muscle (Singh, 1953). As in living muscle, latency relaxation increases with subsequent increase in tension, or decreases.

Effect of other acids.—Other acids like nitric, sulphuric, acetic, also cause contraction of muscle.

DISCUSSION

Strong hydrochloric acid solutions can hardly be considered as physiological stimuli to the contractile mechanism of muscle, yet the effects produced by them on dead muscles resemble the responses of living muscles so closely, that it suggests a close relationship between the two phenomena. Normal contraction, therefore, resembles coagulation of proteins, this resemblance presumably existing in the development of cross linkages in both (Singh, 1954, *b*, *c*).

Other experiments also suggest the similarity between the processes of normal contraction of muscle and coagulation of protiens. The pyloric end of the stomach shows greater tone than the cardiac; the contractile mechanism of the cardiac end can be made to respond with hydrochloric acid, like the contractile mechanism of the pyloric end, if the former is heated to 70° C., that is, if it is more coagulated.

These experiments suggest that the contractile mechanism of unstriated muscle is a variable entity, so that some of the variations in the response, which may be presumed to be due to the excitatory mechanism, are really due to variations in the contractile mechanism. An important variation of the contractile mechanism is its inability to relax, though the excitatory system

may be functioning normally. Absence of relaxation therefore does not exclude the presence of an inhibitory nerve supply or inhibitory receptors on the cell surface.

With increase in initial length, muscle responds by greater tension during contraction; this property is resident in the contractile mechanism, as shown by the effect of hydrochloric acid on heated muscle. An explanation may be offered for this increase of tension (Fig. 16). It may be presumed that points of attraction are located on parts of the folded polypeptide chains these parts are represented diagrammatically as oblique lines. At the ends of each line, there are two points of attraction, represented by empty and dark squares respectively; these squares are on opposite sides of the line, a pair at each end, and exert their attractive forces in opposite directions. It is presumed that the attractive force exerted by each square, empty or dark, exists only on one side of the line, so that in the figure, A_1 attracts A_2, A_3 attracts A_4, and so on. Similarly, B_1 attracts B_2, B_3 attracts B_4, and so on.

When the muscle is stretched, two things happen. First, these lines are straightened out by stretching of the muscle, so that they rotate at their centre and bring the points A_1 and A_2, A_3 and A_4, etc., nearer together, and the points B_1 and B_2, B_3 and B_4, etc., more further apart. If the attraction between the points A_1, A_2, A_3, A_4, etc., is responsible for contraction, then increase in the length of the muscle would increase the contractile force. Secondly, this effect may be counterbalanced by increase in the distance between individual lines as a whole as an effect of stretching, so that the result will depend upon the net effect of these two opposing forces (Fig. 17). Thus it sometimes happens that stretching only decreases the tension produced as a result of stimulation (Singh, 1938).

A very interesting phenomenon takes place in unstriated muscle. Though twitch contraction may be enhanced on increase of initial length tone may decrease; similarly, other tonic contractions, such as produced by potassium, may also decrease (Singh, 1938). This may be explained by the above model if the points B_1, B_2, B_3, B_4, etc., represent attractive forces necessary to produce tonic contraction. These experiments, therefore, suggest that different bonds are formed in the muscle for the production of tone and twitch contraction respectively. Further, bonds for tonic contraction may be of different kinds (Singh and Singh, 1954, *b*, *c*).

The action of hydrochloric acid in reproducing so many phenomena which are produced through the excitatory system suggests that a chemical

substance is liberated as a result of action of a stimulus on the excitatory system. Small concentrations of this substance produce relaxation. It takes time for the substance to reach the maximum concentration required by the stimulus, hence the occurrence of latency relaxation, which being produced whilst the concentration of this hypothetical substance, presumably ATP, is low.

The rate of release of the hypothetical substance which activates the contractile mechanism, depends upon the excitability. If the excitability is increased, the latency relaxation decreases and is ultimately not recordable by ordinary apparatus. Such long duration of the latency relaxation in unstriated muscle suggests that the rate of release of the activating substance is very small compared to that in striated muscle, in which it is comparatively an explosive phenomenon.

The early tension relaxation is of two kinds in unstriated muscle (Singh, 1953). In one kind as the early tension increases, the subsequent tension decreases. In the other kind the latency relaxation and the subsequent tension vary in the same direction. This has been ascribed to a mixture of responses; one is inhibition and the other latency relaxation proper. These results produced through the excitatory system, have been exactly reproduced by the action of hydrochloric acid on the contractile mechanism. It is possible that these are due to the presence of two systems in unstriated muscle, one of which only relaxes, and the other contracts after a preliminary relaxation.

These experiments show that the basis of Starling's law of the heart involves some change in the proteins of the contractile mechanism. Two factors are responsible for increase in the strength of contraction, increase in the initial length and increase in the concentration of the chemical substance acting on the contractile mechanism. The former probably acts by diminishing the distance between the points of attraction in the molecules of the contractile proteins and the latter increases the attractive forces. In heart failure a derangement of both these factors may occur; the treatment of the failing heart would therefore be either to increase the attractive forces or to diminish the distance between them by some modification of the molecular pattern.

SUMMARY

1. Muscles were killed by heat and their contraction studied by the use of strong hydrochloric acid solutions.

2. Though strong hydrochloric acid solutions are anything but a physiological stimulus, yet the responses produced resemble those produced on

the living muscle as the result of a physiological stimulus so closely, that there must be a close relationship between the normal response of the contractile mechanism and that produced by hydrochloric acid.

3. This resemblance between the two phenomena probably depends upon the formation of cross linkages in both.

4. The contractile mechanism of unstriated muscle is a variable entity, so that the response to a stimulus is not only conditioned by the state of the excitatory system, but also by that of the contractile system.

5. The increase in tension produced when the muscle is stimulated with increasing initial length, is also produced by hydrochloric acid on heat killed muscle, so that it is a property of the contractile mechanism.

6. Small concentrations of hydrochloric acid produce relaxation; physiologically weak stimuli produce relaxation of unstriated muscle.

7. A contracted heat killed muscle is relaxed by hydrochloric acid, and a relaxed muscle contracted. This again simulates the physiological response very closely.

8. The sensitivity of the contractile mechanism to hydrochloric acid varies in different parts of the stomach. The cardiac end is most sensitive, and pyloric, the least.

9. The pyloric end does not relax. This also simulates the physiological response.

10. The contractile mechanism has an optimum length for relaxation. This also simulates the physiological response.

11. Hydrochloric acid produces latency relaxation in the heat killed muscle. This latency relaxation closely resembles that produced in living muscle.

12. The mechanism of contraction of unstriated muscle is discussed.

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THE EFFECT OF SPRAYING MCPA (Sodium 2 methyl 4 chlorophenoxyacetic acid) TO SUPPRESS *CYPERUS ROTUNDUS*, LINN.* ON THE YIELD OF WHEAT CROP GROWN IN AN INFESTED FIELD

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[Communicated by Prof. L. S. S. Kumar, M.Sc. (Lond.), A.R.C.S., D.I.C. (Lond.), F.A.Sc., F.N.I.]

Introduction

The reduction in the overall yield of agricultural crops due to the competition offered by weeds is too well known to need further substantiation. It is more so with the infestation of perennial weeds than annuals. One such weed is *Cyperus rotundus*, Linn. commonly known as nut-grass. Because of the presence of 'nuts' or tubers placed at safe distances below the soil surface which are capable of regenerating even when detached from the parent plant, none of the methods adopted hitherto for controlling the weed has been found satisfactory.

Recently experiments with the use of synthetic hormonal herbicides have shown that even an obnoxious weed like *C. rotundus* can be controlled by a judicious application of certain herbicides (Kumar, Solomon and Rao, 1949; Eames, 1949; Burgis, 1949). In the case of a perennial weed like *C. rotundus*, it has been pointed out that the herbicidal method of control is more economical as compared to hand-digging and that the degree of control by the herbicide application is as good as hand-digging. (Arakeri and Patil, 1950). It was therefore thought worthwhile to find out in what way the yield of wheat would be affected by treating the soil for the control of *C. rotundus*.

Material and Method

The field used for the experiment was one which was particularly heavily infested with *C. rotundus*. It was kept fallow during the monsoon and prepared for wheat crop in the usual way in the winter. The field was divided into 48 plots, each measuring 15′ × 15′. Wheat variety Niphad-4 was sown in each plot keeping a spacing of 12″ between rows and 3″ between plants. The crop was irrigated every ten days. The following were the treatments:—

1. Plots with *C. rotundus* .. .. Plots without it (major plot treatment)

* Vernacular names of *C. rotundus*.—*Lavala* (Marathi); *Motha* (Gujerati); *Tungegadde* (Kannada); *Mutha* (Hindi); *Korai* (Tamil); *Gandala* (Telugu); *Motha* (Bengali).

2. Plots treated with the herbicide .. Plots not treated (sub-plot treatment)

3. Different sowing dates, *viz.*—

(*a*) Sown immediately after spraying
(*b*) Sown two weeks after spraying
(*c*) Sown four weeks after spraying
} Sub-sub-plot treatment.

In the case of plots without nut-grass any plants coming up were removed regularly. Spraying was done at the rate of 200 gallons of 0·2% MCPA per acre. A split plot design with four replications was used. All the treatments were randomised for each replication.

The percentage of germination in each of the 48 plots was recorded. When the earheads came to maturity each of the 48 plots was harvested separately and the weights of grain and straw recorded. The data obtained were statistically analysed in order to ascertain the significance of the results obtained.

Table showing the mean of four replications for germination, yield of grain and straw of wheat

Treatment	Germination in %	Yield of grain in lb. per plot	Yield of straw in lb. per plot
Nut-grass, treated, wheat sown immediately after spraying	49·5	1·28	1·78
Nut-grass, treated, wheat sown two weeks after spraying	54·0	0·97	2·47
Nut-grass, treated, wheat sown four weeks after spraying	58·5	1·08	2·55
Nut-grass, not treated, wheat sown immediately after spraying treated plots ..	59·25	0·32	0·73
Nut-grass, not treated, wheat sown two weeks later	58·25	0·20	0·58
Nut-grass, not treated, wheat sown four weeks later	66·25	0·22	0·64
No nut-grass, treated, wheat sown immediately after spraying	54·75	1·75	3·39
No nut-grass, treated, wheat sown two weeks after spraying	58·50	1·53	3·54
No nut-grass, treated, wheat sown four weeks after spraying	75·75	1·25	3·04
No nut-grass, not treated, wheat sown immediately after spraying treated plots	62·75	1·31	2·54
No nut-grass, not treated, wheat sown two weeks later	58·75	1·47	2·79
No nut-grass, not treated, wheat sown four weeks later	72·00	1·70	3·25

Summary of Results

(Yield of grain in lb. per acre)

	Treatment				No treatment				
	S. 1	S. 2	S. 3	Mean	S. 1	S. 2	S. 3	Mean	Mean
Nut-grass	248	188	209	215	64	39	42	48	132
No nut-grass	330	296	242	289	254	284	281	273	281

S. 1	S. 2	S.3	S.E.
224	202	193	19·7

S.E. for the body of the table 39·3

(S. 1, S. 2, S. 3 refer to different sowing dates)

From the analysis of variance obtained for germination of wheat crop, it was evident that the treatment had no effect on germination. However, the third sowing date gave better germination than the first two. This might have been due to climatic conditions. The interactions between sowing dates and the treatments were not significant.

Analysis of variance for yield of grain in wheat showed that the absence of nut-grass resulted in a significant increase in the yield of grain. The increase was as high as 112·8% over the control. Plots treated for suppression of nut-grass showed a significant increase in the yield (34·4%) as compared to those not treated. The different sowing dates did not give significantly different yields; nor were the interactions between sowing dates and treatments significant.

The yield of straw was also significantly greater in plots without nut-grass as compared to those with nut-grass. Plots treated with the herbicide for suppression of nut-grass also showed a significant increase in the yield of straw as compared to the untreated plots. The different sowing dates did not give significantly different yields, nor were the interactions between sowing dates and treatments significant.

From the above it may be concluded that the measures taken to control *C. rotundus* by spraying Sodium MCPA resulted in a highly significant increase in the yield of grain and straw of the wheat crop. Sowing wheat immediately after spraying did not significantly depress the yields.

Summary

Data on the effect of spraying Sodium MCPA to suppress *Cyperus rotundus*, Linn. a perennial weed, on the ultimate yield of wheat crop grown in the area are presented.

Spraying at the rate of 200 gallons of 0·2% spray liquid per acre has shown that the herbicide has no deleterious effect on the germination of wheat.

Spraying for control of nut-grass has resulted in a 34% increase in the yield of grain over the control.

Acknowledgments

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A RAPID COLORIMETRIC METHOD FOR THE EVALUATION OF ORGANIC MATTER OF THE SOILS OF MADHYA PRADESH

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The importance of soil organic matter in relation to soil fertility need hardly be emphasised, specially in subtropical country like India where oxidative decomposition is so rapid. The time to time addition of organic manures of a bulky nature for the maintenance of soil fertility becomes almost imperative and therefore a quick test for the evaluation of organic matter content of the soil becomes an essential part of the rapid methods of soil testing.

A number of such rapid methods have been evolved during the past few years depending upon the action of alkalis, sulphuric acid, potassium permanganate and a mixture of dichromate and sulphuric acid. Of all these methods, modifications of the Schollenberger (1927) dichromate method have proved, so far, to be quick and reliable.

The author has tried some of these modifications, such as the Thomas and Williams method (1936) as modified by the Ontario Agricultural College Department of Soils and the method developed by Khanna, Prasad and Bhattacharya (1949) to see if any of these could be adopted in the case of the soils of the State. The results of these observations are recorded here.

The Thomas and Williams method gives good results with soils containing more than 2% of organic matter but at lower levels the colours obtained range from yellow to orange and are difficult to distinguish for the common eye. Moreover, the standard colours produced with sucrose do not quite match in tinge with those produced by soils of the same organic matter contents. As a majority of the soils of the State contain less than 2% of organic matter the method does not suit well in these cases.

Khanna's method although originally meant for soils with low percentages (Organic C: 0·1–0·7%) of organic matter, was tried to see if it could be modified to suit the Madhya Pradesh soils. Trials made showed that the strength of the dichromate solution and the volume of sulphuric acid used in the original method did not suit these soils and had to be changed

so as to give variations of colour easily distinguishable for soils ranging between 0·7–3% of organic matter. It was observed that 2 c.c. of a 2·5 N dichromate solution with 2·5 c.c. of sulphuric acid (sp. gr. 1·83–1·84) for 1 g. of soil gave good colour variations with these soils. The procedure finally adopted is as follows:—

PROCEDURE

One flat spoonful (roughly 1 gm.) of soil is placed in a hard glass test-tube and to this 2 c.c. of 2·5 N potassium dichromate are added. To the contents of the tube are slowly added 2·5 c.c. of strong sulphuric acid shaking the tube well from time to time. The tube is allowed to remain for 10 minutes with occasional shaking. After the soil settles, 3–4 drops of the upper clear liquid are poured in the depression of a porcelain tile and the colour is matched with the colours of the standard plate.

To prepare a standard colour plate 5 soils of known organic matter content and with variations generally occurring in the soils of the State were used. Colours were developed in each of these soils by the above technique and recorded. The following table gives the results obtained:—

TABLE I

Showing standard colour chart

Sl. No.	Description of soil	Organic matter % Allison's method (C × 1·724)	Description of colour as per Repertoire Decouleurs by Henri Dauthenay	Common colour description
1	Kachhar soil, Harda, Nimar District	2·80	Bright moss green	Bright green
2	Station Garden soil under potato, Chanda	2·01	Sap green	Dirty yellowish green
3	College Farm Soil, Nagpur, behind Mycologist Field Laboratory	1·53	Golden bronze green	Dark brown
4	Field of Shri Bhagwat under Juar, Akola	1·04	Yellow lake	Brownish yellow
5	Soil of a cultivator, Tharsa (Bhandara Tahsil)	0·714	Yellow lake	Reddish Brown

It will be seen from the above table that it is possible to roughly evaluate the organic matter with the help of these colour standards. A bright green colour is obtained in case of soils containing 3% or more of

TABLE II

Showing the comparison of organic matter contents as obtained by rapid colorimetric and the Allison's method

Sl. No.	Description of soil	Organic matter % by Allison's method (C × 1·724)	Organic matter % by rapid colorimetric method
	Soils from cotton and juar area:		
1	Nagpur College Farm soil under cotton	1·39	1·5–2·0
2	Soil from the field of Shri Hoto, Daryapur Tahsil	1·51	1·5
3	Soil from the field of Shri Patil, Ghusar, Akola Tahsil ..	0·78	0·7
4	Soil from the field of a cultivator, Akola	0·91	1·0
5	Chindwara soil	0·78	0·7
6	Orange garden soil, Ubali ..	1·35	1·5
7	Soil from the field of Shri Patil, Pusda, Amravati District ..	1·04	1·0–1·5
8	Soil from the field of Shri Ganorkar, Daryapur Tahsil	1·07	1·0
9	Soil from the field of Shri Hatwa, Tharsa, growing wheat ..	0·67	0·7
	Soils from the wheat area:		
10	Betul Farm soil	0·678	0·7
11	Mariar soil from Hoshangabad ..	0·727	0·7–1·0
12	Heavy clay soil, Narmada Valley	0·689	0·7
13	Soil from Adhartal Farm, Jabbalpur	1·43	1·5–2·0
14	Soil from Kheri Farm, Jabbalpur	0·70	0·7
	Soils from paddy area:		
15	Sindewahi Farm Soil	0·78	0·7
16	Dorsa soil, Raipur	0·66	0·7
17	Wardi soil, Sindewahi ..	1·28	1·0–1·5
18	Soil from the field of Shri Bhadupote, Bhandara District..	0·73	0·7
19	Soil from the field of Shri Ujaone, Bhandara District	1·94	1·5–2·0
20	Bhata soil, Raipur	1·21	1·5
21	Bhata—Matasi soil, Khoka, Drug Tahsil	1·37	1·0
22	Dorsa soil, Raipur	1·27	1·0–1·5
23	Matasi soil, Raipur	1·52	1·5
24	Company Garden soil, Chanda ..	1·19	1·0–1·5

organic matter, a dark-brown for soils with 1·5% and a reddish brown for soils with 0·7% or below. A number of soils with varying organic matter contents, occurring in different parts of the State and under different kinds of crop were analysed by the above process. The above table gives the results of analysis.

It will be seen from the above table that the technique used gives a fairly good evaluation of organic matter to serve as a field test for soils of this state.

As the soils of the State are observed to vary in their C/N ratio within the very narrow limits of 10–11, an estimate of the percentage of nitrogen in the soil could be obtained from the percentage of organic matter as determined by the above process, by dividing by 18 (1·724×10–11). This can be well seen from the following table, which gives the percentages of nitrogen as actually obtained and as calculated.

TABLE III

Showing the percentage of nitrogen as estimated and as obtained by calculating from the organic matter percentage, determined by the quick method

Sl. No.	Soil	Nitrogen % as estimated	Nitrogen % as calculated
1	Nagpur Farm Soil behind Mycologist Field Laboratory ..	·0503	·044
2	Betul Farm Soil	·0744	·081
3	Matasi Soil from Labhandi, Raipur	·0761	·081
4	Soil from Adhartal, Jabbalpur ..	·0719	·079
5	Kabar Soil from Kheri Farm ..	·0483	·039
6	Mariar Soil from Hoshangabad	·0438	·044
7	Chindwara Soil	·0461	·039
8	Station Garden Soil, under potato, Chanda	·0922	·111
9	Sample of Soil from Shri Kelkar, Balaghat	0·404	0·445

SUMMARY

A rapid colorimetric field test for the evaluation of organic matter in soils of the State has been evolved. Two c.c. of 2·5 N potassium dichromate and 2·5 c.c. of strong sulphuric acid for 1 gm. of soil have been found to give a fairly good estimate of the organic matter content. The method works well within the limits of ·7–3% of organic matter, and gives a bright

green colour for soils containing 3% or more of organic matter, a dark-brown for soils with 1·5% and a reddish brown for soils with 0·7% or below.

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MULTIPLE BUDS IN SUGARCANE

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I. Introduction

Dillewijn (1952) gave all types of bud-abnormalities which comprised twin and multiple buds in nodal zone, sometimes decussately arranged and adventitious buds on internodes.

Among the causes of their formation, all the workers (Khanna, 1933, Raghavan, 1951 and Thakur, 1952) agreed that such buds were not inherited. Only Jeswiet[1] (1916) found that in one clone they persisted even after 4 years' repeated vegetative propagation, thus showing their genitical origin. Raghavan thought that some physiological cause involving hormones or effect of minor elements was responsible for them while Thakur was of opinion that primary meristematic cells were likely to protrude out at places more than one to form accessory buds when due to the diminished apical dominance, the development of the terminal bud was retarded. Martin (1938), however, was able to produce galls with buds in them and to bring about the bifurcation of the apical growing point in two out of 21 stalks by injecting insect (*Dræculacephala mollipes*: green leaf-hopper) extract near the growing point.

The present paper puts forward geotropic disturbance in cane stalk as a plausible explanation for the occurrence of multiple buds in some cases.

II. Observations

Sixty-nine stalks belonging to 24 varieties (8 Co canes, 4 indigenous varieties, 11 X-varieties and one B.O. cane) with multiple buds were collected at harvest in February 1951 and 1952. Two-three buds at a node were the most common feature though as many as 5 buds at a node were also noted in X 4668 in which all of them germinated to produce shoots, though somewhat diminished in size (Fig. 1).

In several cases, the abnormality was found associated with the injury to cane-tissues in immediate vicinity caused mostly by insects (borer) which

* Quoted from *Botany of Sugarcane*, by C. Van Dillewijn, p. 24.

brought about the bifurcation of apical growing point in 7 stalks more or less similar to that of Co 213 described by the author earlier (Sharma and Khanna, 1949). In others there was no visible outward manifestation of

TABLE I

Multiple buds in sugarcane due to geotropic displacement of stalk

Variety	Stalk No.	No. of nodes	Multiple buds on nodes	Geotropic curvature at nodes
Co 663 ..	1	27	XVI	XV-XIX
	2	18	XII-XVI	XI-XVIII
	3	22	XV, XVI, *XVII**, XIX	XV-XIX
Co 682		19	XII	XI-XIII
Co 781†		23	XI, XIII, XV, XIX	X-XIII
Co 858†		29	XV, XVII	XIV-XVIII
X 4199 ..	1	22	XII	X
	2	29	XI, *XII*, XIII	XI-XIII
X 3451†		18	X, XII, XIV	X-XIV
Co 650† ..	1	15	VI, VIII	..
	2	15	IV-VI, VII, IX	..
	3	25	*XV*, XVI, *XVII*, XVIII	..
Co 682†		18	XVII, X	..
X 4199† ..	1	20	XII, XIV, XVI	..
	2	19	XIII, XV	..
X 4351†		16	II, IV	..

* Figure italicised means presence of 3 buds, there being 2 in the rest.
† Multiple buds are present on alternate nodes.

any disturbance and the formation of two or more buds could not be ascribed to any precise cause, though propensity of certain varieties to form multiple buds more frequently than others could not be excluded, as would appear from the following:

Co 663	8	stalks	
X 4199 and X 4351	7	„	each
Co 629 and Co 650	6	„	„
B.O. 24, Co 682, Khelia and Sonnabile ..	4	„	„
Co 453	3	„	„
X 4668 and X 4692	2	„	„
Co 331, Co 654, Co 729, Co 776, Co 781, Co 858, X 3928, X 4421, X 4604, X 4766, Hadda and Pultikhazee	1	„	„

There were however 9 stalks (Table I, Fig. 2) in which buds were formed in the curved portion. In the case of Co 663 (3), X 4199 and X 4351, the abnormal-bud zone coincided with the portion of the stalk which showed curvature. In the other 5 cases, the former began at the next higher node, and in the 6th after 2 nodes.

Another interesting observation was that in 3 of them namely Co 781, Co 858 and X 4351, multiple buds were formed only at alternate nodes. This skipping of nodes was all the more remarkable in Co 781 in which though the disturbance affected 9 nodes (XI-XIX), more than one bud was formed at XI, XIII, XV and XIX.

Besides these 3 stalks, 7 others were noted to have abnormal number of buds at alternate nodes. In Co 650 (3), 3 buds were formed on XV and XVII nodes there being only 2 at XIV and XVI.

III. DISCUSSION

From the observations given above, it appears that multiple buds are formed in portions of some stalks which show geotropic curvature while in others their development followed a certain pattern. It was only in 3 cases (Co 781, Co 858 and X 4351) that the 2 features were found together. A plausible explanation in support of which no experimental data are adduced for the present, is given below to account for these observed facts.

In a sugarcane stalk which normally remains more or less erect, growth hormones formed in apical meristem, will tend to accumulate when it assumes a horizontal position as was shown to be the case for vertical organs like *Avena* coleoptile by Dolk*[2] (1929), root-tips of *Vicia faba* by Boysen Jensen[2] (1934) and a segment of *Lupinus* hypocotyl by Dijkman[2] (1934). In addition to this, geotropically stimulated nodes are also likely to manufacture growth substances themselves like the mature ones of *Triticum*, *Secale*, *Hordeum*, *Setaria* and *Lolium* (Schmitz,[3] 1938).

This high concentration of growth substances in the nodal region is likely to stimulate the division of the cell (growth primordium of a bud) as was pointed out by Jost[4] (1935 *b*) in the pith of *Vicia faba*, the main roots of *Lupinus*, etc. Earlier Popoff[4] (1933) reported that dilute concentrations of growth substances obtained from coleoptile and other parts of *Zea* seedlings promoted *inter alia* cell division in *Euglena gracilis*. The stimulation of cell division by ether-soluble thermostable extract from orchid pollen

* Quoted from *Growth Hormones in Plants*, by P. Boysen Jensen, English Translation by G. S. Avery and P. R. Burkholder, McGraw-Hill Book Company Inc., 1936, **2**, p. 5; **3**, p. 197; , p. 103.

and urine in the stems of *Coleus* and *Tradescantia* (Laibach, Nai and Muller, 1934), by 3-indole-acetic acid in the epicotyl of *Vicia faba* (Laibach and Fischnich[4], 1935 *a*) and by purified auxin *a* and 3-indole-acetic acid in the cambial region of upper ends of decapitated *Helianthus* seedlings (Snow[4] 1935–36) may also be cited in this connection. Finally Laibach and Mai[5] (1936) described malformations of leaves and buds caused by repeated application of concentrated auxin paste to the growing point.

The skipping of nodes appears to lend further support to the explanation given above. Though the exact mechanism of longitudinal transport of growth substance is not completely understood (Boysen Jensen, 1936), one of the components of vascular bundle, phloem according to Haberlandt[6]† (1913) and Cooper (1936) and xylem according to Hitchcock and Zimmerman[7] (1936) is the path-way where considerable distances are involved. Avery[7] (1935) showed that auxin moved exclusively through the vascular bundles in the leaves of *Nicotiana* and *Malva*. Therefore in the case of a prostrate sugarcane stalk, auxin which is likely to accumulate in the lower half of its cross-section and to travel along vascular bundles of that portion only, will effect bud primordia situated in the immediate vicinity of its passage while those away from it will not be, in any way, influenced. Now if, a cane falls along its median‡ diameter, bud-primordia nearest the earth would be stimulated by higher concentrations of auxin while those away from the earth will be beyond its reach. If, on the other hand, the cane-stalk lies along its lateral diameter, bud-primordia on all the nodes will be more or less equidistant, and thus affected in the same manner. In view of the fact that vascular bundles in some varieties extend over 6–8 nodes, it is interesting to note that in Co 781 stalk, double buds were present on nodes XI, XIII, XV and XIX while nodes XII, XIV, XVI, XVII and XVIII were skipped over.

As regards the 7 other cases of skipping where no curvature was noted, it is just possible that uneven lighting of cane stalks coupled with some other environmental factor might have brought about a higher concentration of auxin near the median diameter and thus produce double buds in erect canes.

IV. Summary

1. The non-genitical nature of bud-abnormalities of various types has been universally admitted except in one case reported by Jeswiet in which

† Quoted from *Phytohormones*, by F. W. Went and K. V. Thimann, MacMillan Company, New York, 1945, **5**, p. 248; **6**, p. 17; **7**, p. 91.

‡ The diameter of a cane stalk which passes through the vertical plane having buds at alternate nodes is by convention known as its median diameter and that at right angle to it as lateral diameter.

triple buds persisted in a clone even after vegetative propagation for 4 years. Their occurrence is ascribed to hormones, minor elements or diminished apical dominance by different workers.

2. Out of 69 stalks belonging to 24 varieties with multiple buds, several had insect injury in immediate neighbourhood while in many others no outward manifestation of any disturbance was descernible. Greater number of stalks found in one variety than in the other, indicates a varietal propensity towards such malformations.

3. In 8 cases belonging to 5 varieties, occurrence of multiple buds was found associated with curvature in that portion of the stalks while in 3 of these and 7 others such buds were formed on alternate nodes.

4. Geotropic disturbance of stalk is discussed as a plausible explanation to account for these observed facts.

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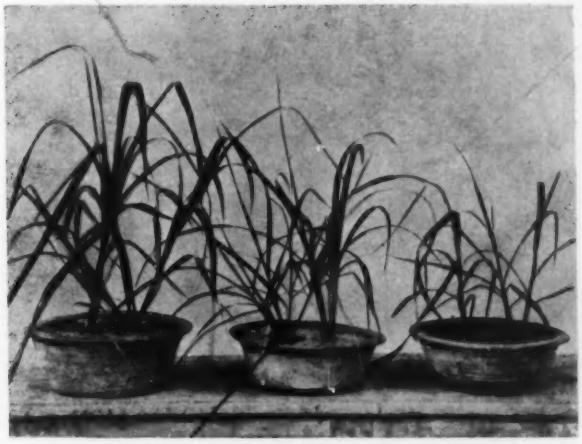
X-MEN



a

FIG. 1. Germination of multiple buds (2–3 at a node).

a. All the five buds at a node in ×4668 germinated to produce shoots though diminished in size.

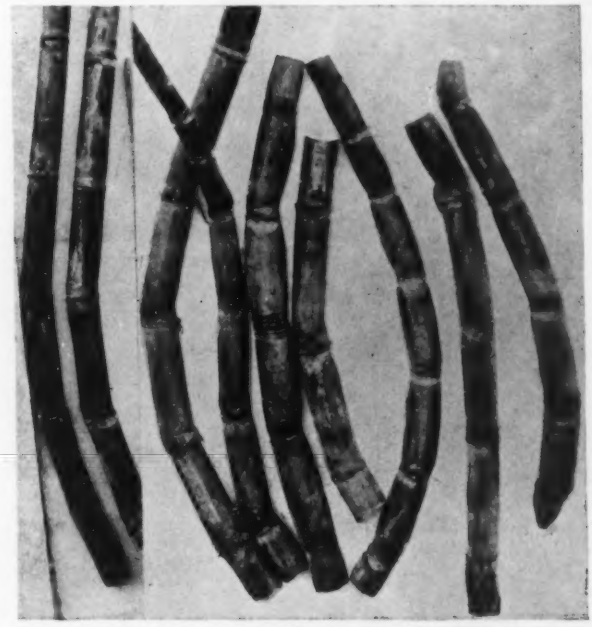


FIG. 2

ON A NEW CESTODE, *OOCHORISTICA HEMIDACTYLI* FROM THE COMMON WALL LIZARD, *HEMIDACTYLUS FLAVIVIRIDIS* RUPPELL

By G. N. Johri

(*Department of Zoology, Lucknow University*)

Received September 13, 1954

(Communicated by Dr. M. B. Lal, F.A.SC.)

A large number of different lizards were examined from various localities for the cestode parasites and several interesting forms were obtained. In the following account a new form is being described.

Family *Anoplocephalidæ* Fuhrmann, 1907.

Oochoristica hemidactyli n. sp.

(Figs. 1–4)

One complete worm and two strobilæ were obtained from the small intestine of a common wall lizard, *Hemidactylus flaviviridis* Ruppell, out of several dozens examined at Banaras and Lucknow. Maximum length 49 mm., maximum width 0·697 mm. Scolex measures 200 μ in diameter and is followed by a short neck. The suckers are almost circular and measure 80 μ in diameter. The proglottids of the anterior region are equal in length and breadth while the posterior ones are longer than broad. Dorsal and ventral longitudinal excretory canals are respectively 0·025 and 0·75 mm. wide. Genital pores irregularly alternate and situated near the anterior fourth of the proglottid. Testes postovarian, in two distinct lateral groups and 19–30 in each proglottid. Each testis measures 0·045·0–07 mm. in diameter. The vas deferens can be seen at the level of the ovary and it gradually increases in width towards the genital pore. The cirrus sac measuring 280–300 $\mu \times$ 40–70 μ extends upto the ventral poral longitudinal excretory canal and about one-seventh across the proglottid. A distinct cirrus is not visible. Genital ducts pass dorsal to poral excretory canals. Ovary is a median, bilobed structure, located in the anterior half of the proglottid and measures 0·96 mm. across its widest part. The vagina is almost straight and opens into the genital atrium posterior to the cirrus sac. A receptaculum seminis is not distinctly visible. A shell gland mass is present behind the ovary. The vitelline gland is irregular in shape and measures 0·31 mm. in diameter. Uterus transverse, tubular which eventually breaks up into

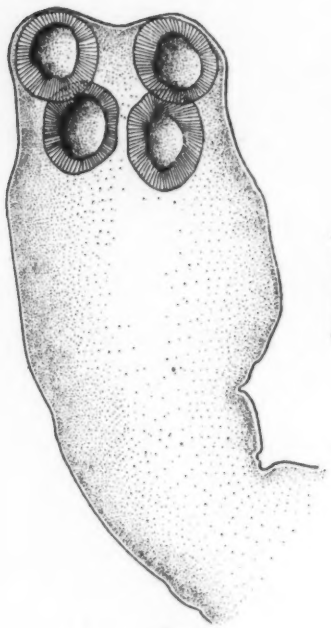


FIG. 1

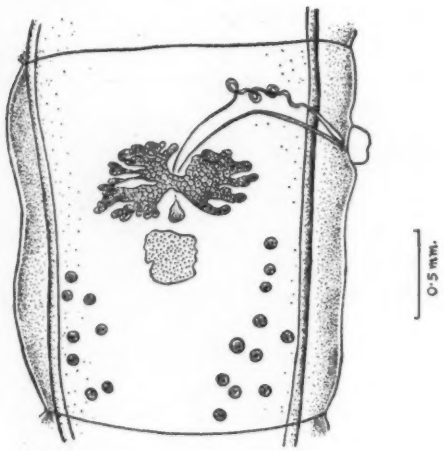


FIG. 2

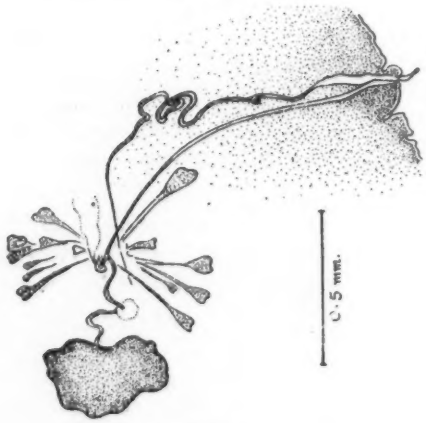


FIG. 3

FIG. 4

Oochoristica hemidactyli n. sp.

FIGS. 1–4. Fig. 1. Scolex. Fig. 2. Mature proglottid. Fig. 3. Showing disintegration of the ovary. Fig. 4. Egg.

more or less evenly distributed capsules, each containing one egg. Eggs measure 46–65 μ and onchospheres 28–30 μ in diameter. The embryonic hooks measure 11–13 μ in length.

Of all the species of genus *Oochoristica* Luhe, 1898, so far known, the present form closely resembles *Oochoristica pennsylvanica* Chandler and Melvin, 1951; *Oochoristica khalili* Hamid, 1932; *Oochoristica rostellata*

Zchokke, 1905 and *Oochoristica tuberculata* Rudolphi, 1819, but it can be easily distinguished from all these by the small size of its body and correspondingly smaller scolex and suckers. It can be further distinguished from *O. khalili*, *O. rostellata* and *O. tuberculata* by the disposition of its testes in two distinct lateral groups. It also differs from *O. khalili* and *O. rostellata* in having lesser number of testes, and from *O. pennsylvanica* by the much larger size of its cirrus sac which extends upto the ventral longitudinal excretory canal. Form *O. rostellata*, the present form also differs in the absence of a receptaculum seminis and from *O. khalili* in having much smaller eggs. The present form is, therefore, regarded as a new species which is named *Oochoristica hemidactyli* n. sp.

The author is highly grateful to Dr. M. B. Lal, for his kind guidance in the preparation of the present paper.

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ON A NEW CESTODE FROM THE INDIAN COBRA, *NAIA NAIA* LINN.

By G. N. Johri

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Received December 6, 1954

(Communicated by Dr. M. B. Lal, F.A.SC.)

About a dozen snakes from Banaras and Lucknow were examined for helminth parasites and two of them harboured some cestodes which on study were found to represent the genus *Ophiotænia* La Rue, 1911.

Ophiotænia n. sp.

(Figs. 1–3)

Six complete worms with gravid proglottids, several strobilæ and numerous detached proglottids were obtained from the hosts. Length 110–180 mm. Breadth 1·4–1·55 mm. Scolex large, slightly wider than the neck and measures 200–210 $\mu \times$ 280–370 μ. Suckers circular, directed outwards and measure 120–135 μ in diameter. A fifth sucker is absent. Neck long, measuring about 7 mm. in length and 0·35 mm. in width at its broadest portion. The first few segments immediately behind the neck region measure 0·5–0·57 mm. $\times$ 0·75·0–79 mm. The mature segments are roughly square measuring 0·97–1·32 mm. $\times$ 1·24–1·4 mm., while the gravid ones are longer than broad and measure 2·9–3·8 mm. $\times$ 0·85–0·90 mm. The musculature is weakly developed. Transverse sections reveal a band of longitudinal muscle fibres near the lateral margins besides the circular and the subcuticular layers. The genital pores irregularly alternate and are situated at the middle of the lateral margins of the segments or slightly ahead. The genital atrium is a simple depression. The cirrus sac oval or elongated, measuring 410–450 $\mu \times$ 140–50 μ, extends upto about one-third across the proglottid and passes in between the excretory ducts. Cirrus is slender, unarmed and coiled. The testes circular or ovoid, distributed in two lateral groups and 31 or 32 in number per segment of which about half are usually on the poral side. They measure 0·06–0·10 mm. in diameter. The vagina is usually in front of the cirrus sac but it may be behind it as well. It is much coiled posteriorly behind the ovarian isthmus and presents an enlargement near the genital pore. The ovary is a bilobed structure extending upto the ventral excretory canals and measures 1 mm. across. There are 15–17 uterine

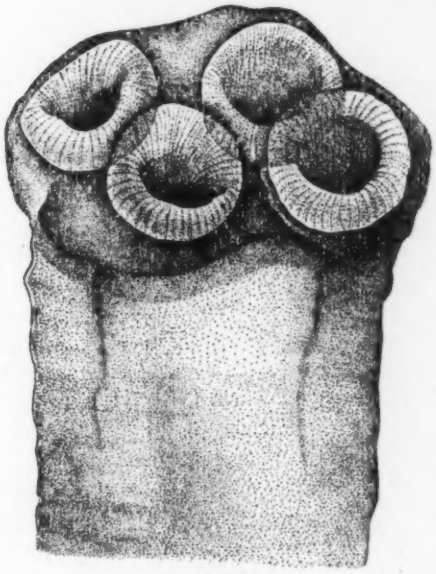

FIG. 1.

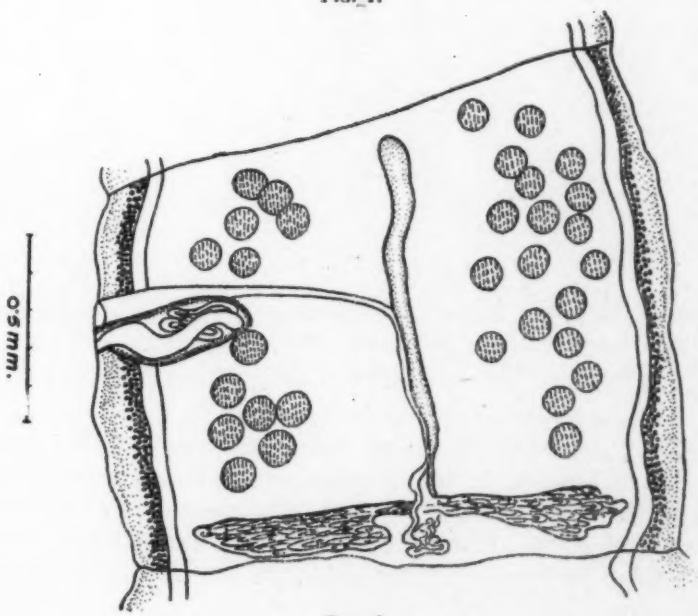

FIG. 2.

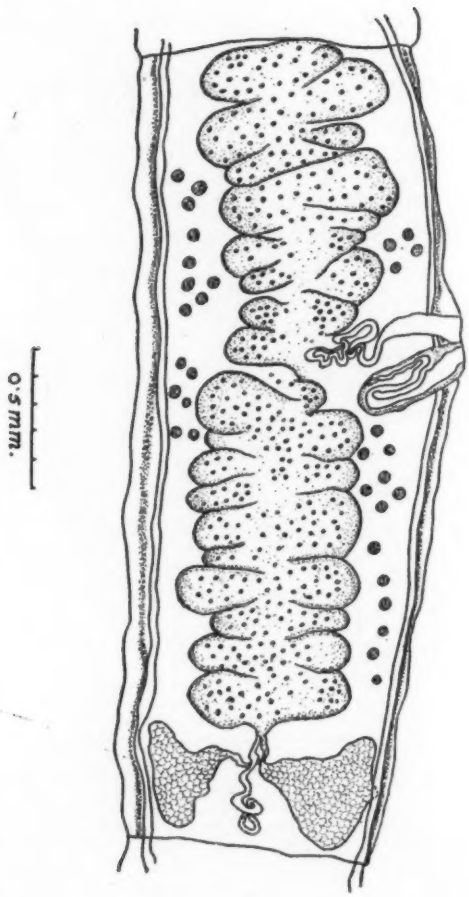

FIG. 3.

pouches on each side. The vitelline glands are distributed in two bands along the lateral margins of the segments. The eggs measure 24–26 $\mu \times 28$–30 μ and oncospheres 10–15 μ in diameter. The hooks are about 5 μ long.

Of all the species of the genus *Ophiotænia* La Rue, 1911, the present form closely resembles *O. saphena Osler*, 1931 and *O. nankingensis* Hsu, 1935. It can, however, be distinguished from both these species by its testes which are far less in number, a feature which is unique for all the known species of the genus. It can be further distinguished from *O. saphena* by the absence of the fifth sucker and also by the size of its cirrus sac which is

much larger in relation to its smaller body size. From *O. nankingensis* also it can be further distinguished by its uterine branches which are less in number and also by the smaller size of its suckers. It is, therefore, regarded as a new species and named *Ophiotænia indica* n. sp.

The author is highly grateful to Dr. M. B. Lal for his valuable criticism and guidance in the preparation of this paper.

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STUDIES IN THE INDIAN AQUATIC FUNGI*

II. On the Occurrence of Two Species of Ancylistes in Closterium

By (Miss) R. John

(Department of Botany, Lucknow University)

Received September 10, 1954

(Communicated by Dr. S. N. Das Gupta, F.A.SC.)

Historical

The genus Ancylistes was established in 1872 by Pfitzer.[12] It was founded on a single species *A. closterii* which was parasitic on *Closterium acerosum*. In 1895 Fritsch[9] added another species *A. cladocerarum* parasitic in rotifers but this is very imperfectly known and was later on excluded from the order Ancylistales. In 1897 Beck[1, 2] included a third species *A. Pfeifferi* in this genus. In 1925 *A. Miurii* was described by Skvortzow[17] but this is a very doubtful species. Recently in 1949 Couch[5] added a new species *A. Netrii* parasitic in a saccoderm desmid, *Netrium digitus*. Of these 5 species described *A. cladocerarum* and *A. Miurii* have been regarded as invalid. At present therefore, there seem to be only 3 valid species of Ancylistes, *A. closterii*, *A. Pfeifferi* and *A. Netrii* of which the two former ones are parasites of Closterium species and the last is parasitic in Netrium.

Previously, in 1858, Schenk[13] had described the genus Myzocytium. In 1859 he described Lagenidium and Achlyogeton.[12, 13] In 1892 Fischer[8] included these 3 genera along with Resticularia and Ancylistes in the family Ancylistaceæ of the order Micochytridineæ. Schroter in 1893[16] made the order Ancylistineæ which contained 2 families Lagenidiaceae and Ancylistaceæ. Achlyogeton, Lagenidium and Myzocytium along with a new genus Rhizomyxa was placed in the Lagenidiaceæ and Ancylistes and Resticularia were placed in Ancylistaceæ. Other genera of uncertain position like Protascus and Lagenidiopsis have also been given a place in the order Ancylistales but their inclusion has been controversial.

In 1938 Miss Berdan[3] published the most complete account of the genus Ancylistes so far known. While collecting desmids around London, Canada, she came across one species of Ancylistes (*A. Pfeifferi*) in dead specimens of Closterium species. Subsequently she collected *Closterium striolatum* from the bottom of a stagnant pool in Chapel Hill, North Carolina and found *A. closterii* parasitic in it. At a later stage *Closterium areolatum*

* Contribution from the Department of Botany, Lucknow University New Series, No. 11.

from the same pond was found to be infected by *A. Pfeifferi*. In course of the investigation of the species of Ancylistes she discovered that the external hyphæ of the parasite when in contact with a closterium acted as infection tubes whereas in the absence of the host they functioned as conidiophores producing dark glistening spherical conidia which are forcibly discharged. The conidium after discharge falls back into the water and forms either a secondary conidium or a germ tube which may act as a conidiophore or an infection tube depending on the availability of the host. Thus it became clear that Ancylistes reproduces asexually by the conidia which are forcibly discharged from the conidiophores as in the Entomophthorales and that the infection was not by zoospores as in the Ancylistales. On the basis of this discovery she revised the genus Ancylistes and removed it from the Ancylistales to the Entomophthorales. With the elimination of the type genus Ancylistes the order Ancylistales was no longer tenable. Therefore in 1939 Karling[10] raised the family Lagenidiaceæ to ordinal rank. Sparrow[18] then included the Olpidiopsidaceæ and Sirolpidiaceæ in this order along with the Lagenidiaceæ. So at present the new order Lagenidiales includes the members which belonged to Ancylistales, namely Lagenidium, Myzocytium and Achlyogeton originally discovered by Schenk in 1858 and 1859.

Introduction

In course of collection of the Aquatic fungi from tanks and ponds in the vicinity of Lucknow the author came across a species of Closterium infected with Ancylistes. The first collection of this fungus was made in February 1952. Some rosaceous fruits were placed as baits in a number of tanks in the Botany Department Garden of Lucknow University. After 3 weeks these baits were collected and brought to the laboratory for studying the possible fungi present in them. Most of these yielded species of Blastocladia some of which have been reported in a previous paper.[7] One of the tanks contained some Spirogyra and the baits which were at the bottom of this tank were found to be covered with a coat of yellowish green sediment. A small quantity of the sediment was mounted in glycerine on a slide and examined under the microscope. The sediment was found to be Spirogyra filaments in various stages of disintegration along with a large number of a species of Closterium which were entangled in the filaments. Some of these Closteria were green and healthy and others were disintegrating. The latter proved to be heavily infected by a fungus. Because of its characteristic multi-cellular thallus and the numerous infection hyphæ there was no difficulty in identifying the fungus as a species of Ancylistes. More of the sediment was then collected from the bottom of the tank and preserved along

with the baited fruits for further examination and study. Attempts were made after 3 weeks to collect the fungus again but by that time the closterium had disappeared from the tank. Since then periodical collections from the same tank and from other places so far has failed to show the presence of Ancylistes although closterium was abundant at certain times. Therefore, this paper is based on the observations made from preserved material only. In the meantime attempts are being made to collect living material and study the various phases of the life-history.

METHOD

The material collected was first preserved in Picro-formol. After about 18 hours it was washed in 50% alcohol and finally preserved in 70% alcohol.

For detailed study a small amount of the material was carefully teased out under binocular microscope in order to separate the infected closterium from the algal filaments with which these remained entangled specially when the infection tubes of the parasite were plentiful. During the process of teasing many of the closteria broke into bits and only relatively few of the infected ones could be obtained unbroken. The closterium free from the extraneous algæ was then transferred to a drop of 70% glycerine by means of a capillary tube and a permanent glycerine mount was made in the usual manner. Most of the closteria were examined unstained, a few, however, were stained with eosin in 70% glycerine or with cotton blue in order to obtain a clearer demarcation. These were studied under bright field as well as phase contrast microscopes.

A careful examination of a number of preparations thus made showed that about 50% of the closteria were normal healthy and free from infection whereas the other 50% were under different stages of infection by Ancylistes. Among the infected ones about 10% showed the early developmental stages of the parasite while the great majority showed advanced stages.

MORPHOLOGICAL CHARACTERS

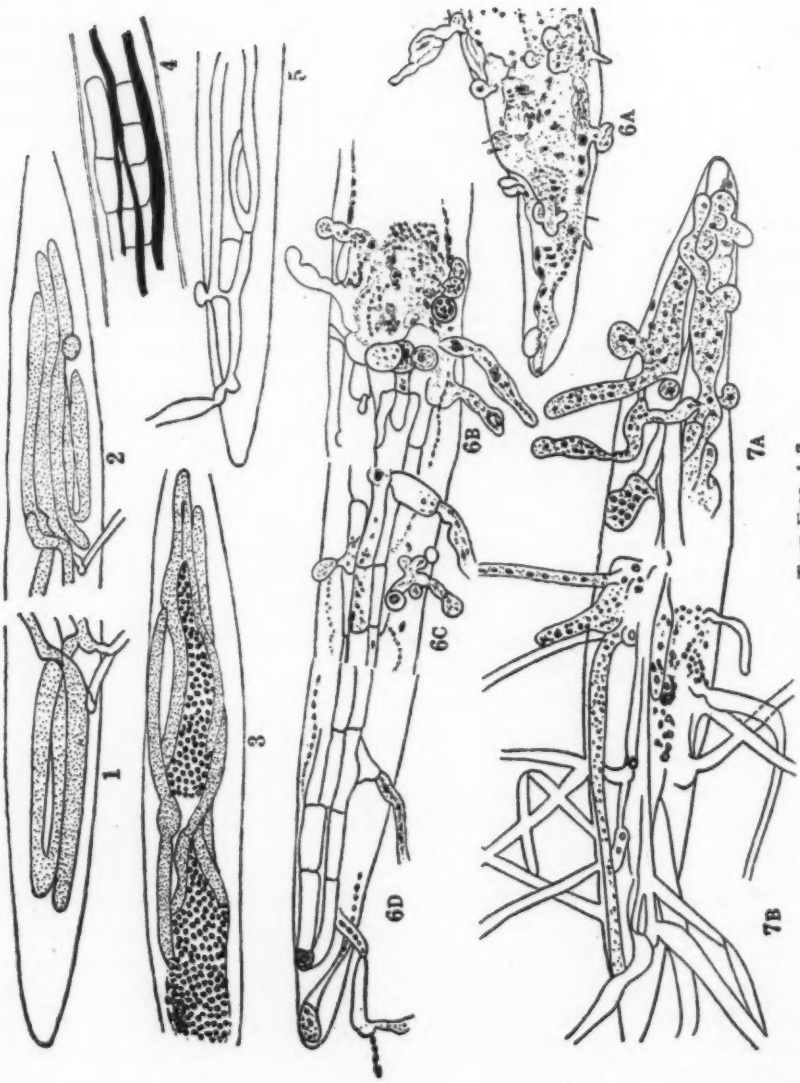
In course of the investigation it was found that the nature of the parasite in a few of the closteria were different from that in the majority. These differences proved to be of specific nature as given by Miss Berdan and other workers. They were then identified as *A. closterii* and *A. Pfeifferi* based on the characters of the infection tubes appressoria and intramatrical mycelium. The vast majority of the closteria were infected by *A. closterii* while only a very few of them contained *A. Pfeifferi*. Careful examination was made to find out if the two parasites infected the same host species or if the closteria were different. No specific differences could be detected

and it seems therefore that the 2 species of Ancylistes parasitise the same species of closterium. The fact that only relatively very few closterii were infected by *A. Pfeifferi*, and these were at the early stage of infection, might indicate that *A. closterii* which showed most of the developmental stages had been flourishing and it is only towards the declining stage of the epidemic that *A. Pfeifferi* had made its appearance and established itself.

A. closterii.—The closteria which were parasitised by *A. closterii* showed various stages of infection while the one containing *A. Pfeifferi* showed only the early stages. Therefore the morphological characters of *A. closterii* could be studied in greater detail than those of the other species. From a careful study of the specimens showing early as well as later stages it has been possible to give an almost complete account of the life-history.

In the early stages of infection the thallus of the parasite is narrow, tubular and non-septate (Text-Figs. 1, 2 and 3 and Plate V, Fig. 1). At the end of the closterium the thallus curves round and goes back in the opposite direction (Text-Fig. 1). In some 2 or 3 young thalli were found to be lying parallel to each other (Text-Fig. 2), a few others showed a globular mass of protoplasm just inside the host which is perhaps the discharged contents of the infection hyphæ forming the beginning of infection (Text-Fig. 2). According to Miss Berdan the contents move from the place of infection towards the centre of the host and attack the nucleus. However, it has not been possible to find out whether the growth of the thallus started from the place of infection or from the nucleus in the centre of the host. When the thallus has attained its full length it becomes wider and afterwards septation starts (Text-Figs. 4, 5 and Plate VII, Fig. 5). The cells swell up in the middle leaving the ends constricted. At maturity the mycelium consists of 30–40 cells which are at least twice as thick as the young thallus (Text-Figs. 11 and 12).

When mature each cell of the thallus sends out one external hypha which may act either as an infection hypha or as a conidiophore. These hyphæ arise as small swellings usually from near the septa and grow towards the cytoplasmic membrane of the host lining the cell wall against which they press and swell. From the end of the swelling papillæ penetrate the wall and grow out as external hyphæ (Text-Figs. 7 A and 7 B and Plate V, Figs. 2; Plate VI, Figs, 3 and 4). These may be few or profuse in number, some extending for a considerable distance outside the host wall (Text-Fig. 7 B and Plate VIII, Fig. 7). At the end of a few of these hyphæ conidia were observed which are hyaline and thin-walled, but no secondary and tertiary conidia were present (Text-Figs. 8 and 9).



Text-Figs. 1–7

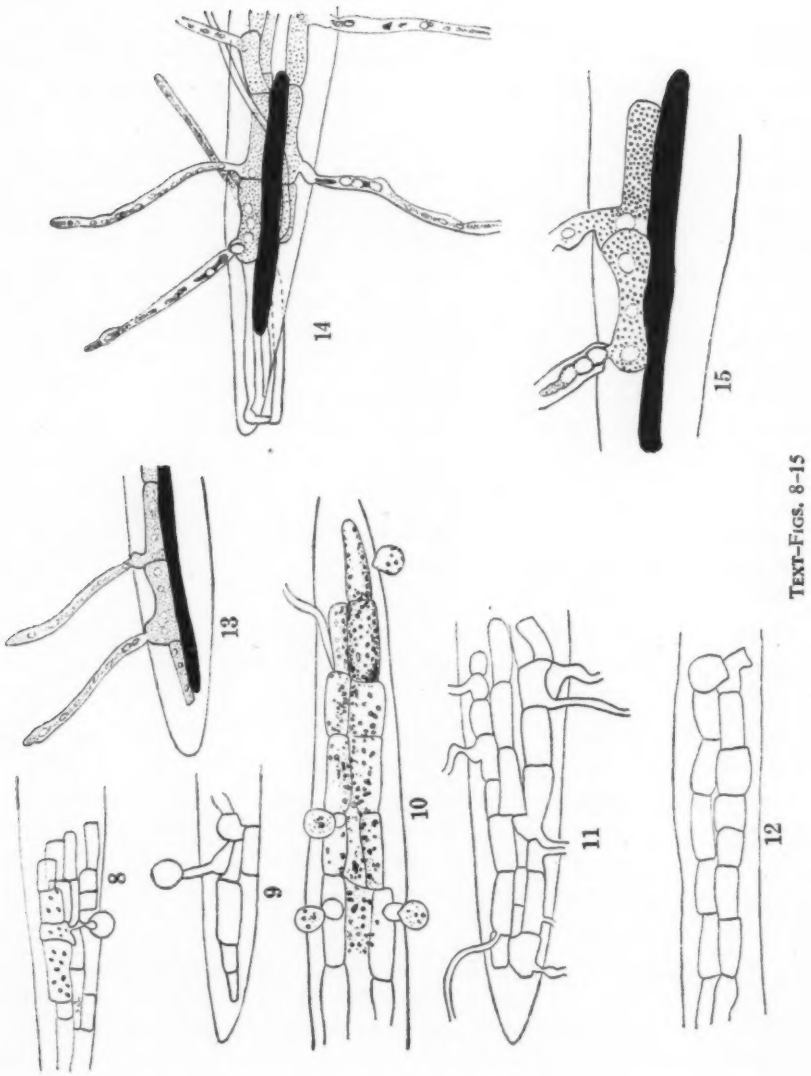
The structure of the thallus and the external hyphæ in the specimens examined so far are essentially similar to those described by previous workers. However, in some of the specimens the author found certain features of interest which have not been recorded so far. The most outstanding among these peculiarities is the presence of certain protuberances at various stages of emergence from the host cell. These generally assume various shapes, being thick, club-shaped, rounded, lobed or globular structures. The morphological nature of these protuberances is not clearly understood but these appear to be the precursors of the external hyphæ. This will be clear from Text-Figs. 7 A and 7 B, which show two-halves of the same closterium where 7 A contains the ordinary mature, thin, tubular external hyphæ and 7 B, the protuberances. These are also seen in Plate VI, Figs. 4; Plate VII, Figs. 5, 6 and Plate VIII, Fig. 7. Text-Figs. 6 A, B, C and D show a closterium where the major part contains protuberances while only one end shows a few ordinary external hyphæ.

The second interesting feature is that in one specimen three conidia-like structures seem to be borne directly by the cells of the thallus and not by conidiophores (Text-Fig. 10, and Plate VII, Fig. 5). At one end of the closterium an external hypha is also seen but it does not bear any conidia. Perhaps in these cases the conidiophores are extremely short.

No specimen has been obtained which show the presence of typical stages of conjugation and resting spore formation, but in several cases round structures were found inside the host cell which are somewhat similar to resting spores (Text-Fig. 7). These have thick-walls and are filled with cytoplasm.

A. Pfeifferi.—There is at present only a few slides of this species. Although the resting spores are not present in this specimen, the character of the cells of the intramatrical mycelium, the infection tubes and the appressoria strongly resemble *A. Pfeifferi.* Therefore, it is provisionally identified as the same.

The earlier development of this species is the same as *A. closterii* but the cells when they are fully mature are quite characteristic in producing enlargements along the hyphæ and joint-like bulges near the septa which makes the cell femur-shaped (Text-Figs. 13 and 14). The cytoplasm in this species is more granular and the vacuoles are very conspicuous. Also, the exit hyphæ are formed as broad outgrowths one from each cell near the septa and they get constricted just before emerging from the host wall (Text-Fig. 15). The appressorium in this species is like a flat plate and is



TEXT-FIGS. 8-15

clearly seen (Text-Fig. 13). As stated before the later stages were not present in the specimens. Attempts are being made to collect and study this fungus more thoroughly. In the meantime we have to depend on the above-mentioned characters for the identification which is provisional. Both *A. closterii* and *A. Pfeifferi* are here recorded for the first time from India.

Description

Ancylistes closterii Pfitzer.—Intramatrical mycelium 5–7 μ wide in the young stage. Cells swollen in the middle and constricted at the ends after septation, 10–15 $\mu \times$ 20–28 μ, filled with highly refractive granules. External hyphæ one for each cell usually formed at adjacent sides of a cross wall, the mature ones thin and tubular 6–8 μ wide and 60–230 μ long, and the young ones showing different types of protuberances. Conidia hyaline, spherical, 11–15 $\mu \times$ 12–16 μ. End of the conidiophore swollen, forming a columella which projects into the base of the conidium. End cell of infection tube swollen 8–12 μ. Appressorium barely discernible. Conjugation not observed. Resting spore smooth, brown, thick-walled, spherical, 11 $\times$ 12 μ filled with refractive globules. Germination not observed.

Ancylistes Pfeifferi Beck.—Intramatrical mycelium irregular. Cells enlarged at the free ends, femur-shaped, 8–10 μ in the centre, 15–17 μ at the ends and 35–45 μ long. Segments with several vacuoles and highly granular cytoplasm. External hyphæ 5–8 μ broad and 90–200 μ long. Appressorium disc-like and clearly seen. Conidia, conjugation and resting spores not observed.

Acknowledgment

I wish to express my gratitude to Prof. S. N. Das-Gupta for his continued help and guidance throughout the course of this work.

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EXPLANATION OF TEXT-FIGURES

TEXT-FIGS. 1–12. *A. closterii.* Figs. 1, 2 and 3. Closteria showing early stages of infection of *A. closterii*, ×770. Figs. 4 and 5. Thallus of *A. closterii* showing septation, ×770. Fig. 6. A, B, C and D. Various parts of one closterium showing the thallus of *A. closterii*, ×770. Fig. 7 A. Part of a closterium showing thallus of *A. closterii* with various types of protuberances, ×770. Fig. 7 B. Another part of the same closterium in which the parasite shows normal external hyphæ. Figs. 8 and 9. Short external hyphæ of *A. closterii* bearing conidia, ×650. Fig. 10. Thallus showing conidia-like structures without any conidiophores, ×770. Figs. 11 and 12. *A. closterii*, showing old empty cells, ×770.

TEXT-FIGS. 13–15. *A. Pfeifferi*, Fig. 13. Part of closterium showing the characteristic femur-shaped cells of *A. Pfeifferi*, ×650. Fig. 14. General view of *A. Pfeifferi* with external hyphæ and appressoria, ×770. Fig. 15. Magnified view of the femur-shaped cells of *A. Pfeifferi*, ×1,000.

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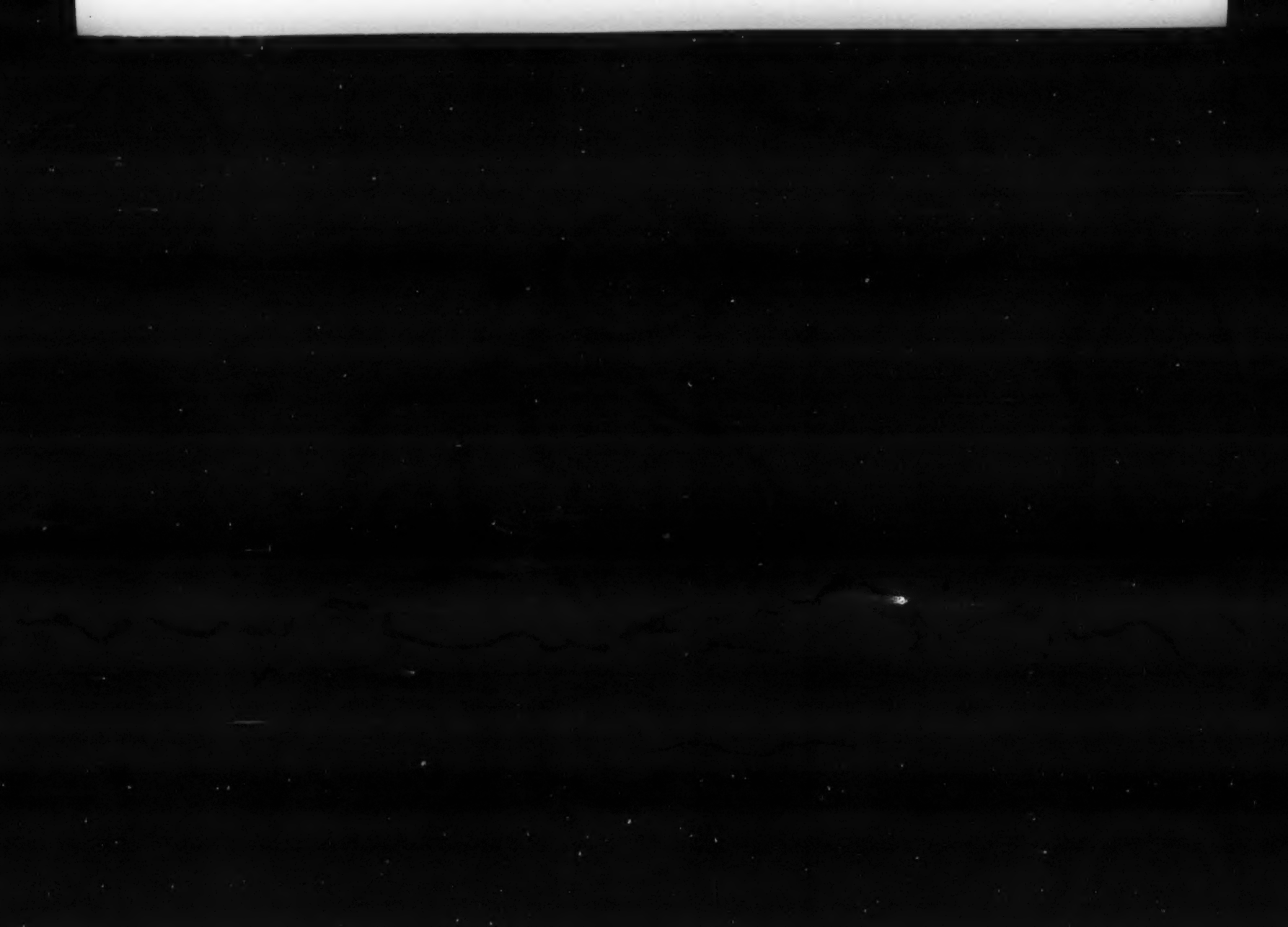

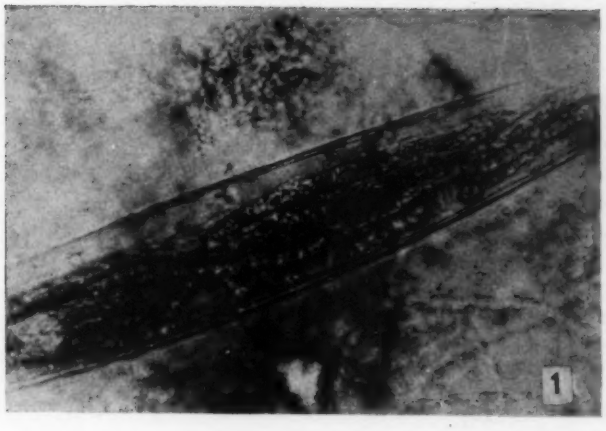
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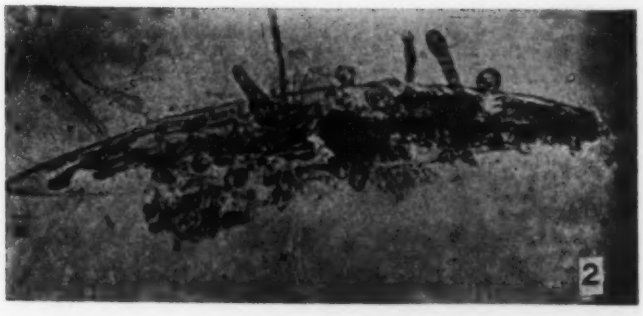
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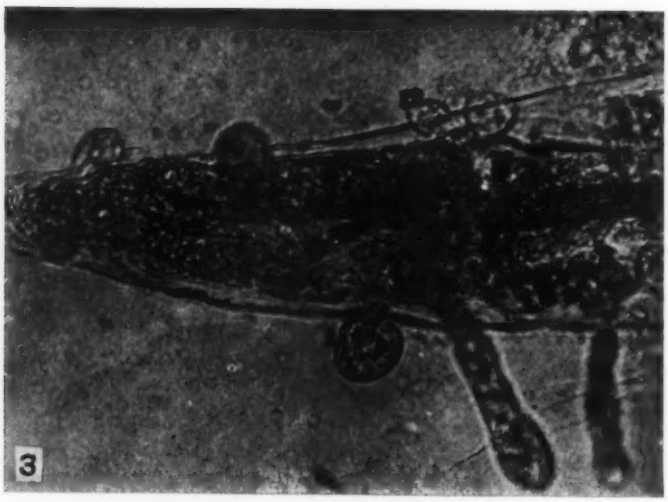
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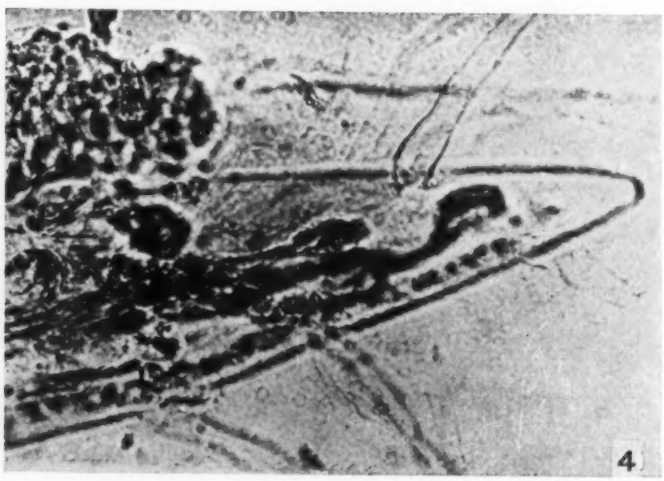
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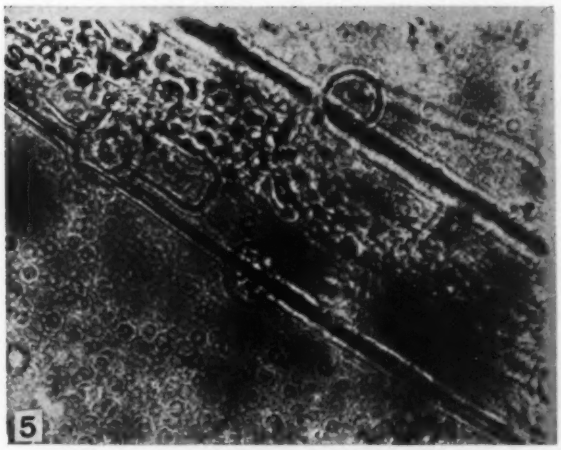
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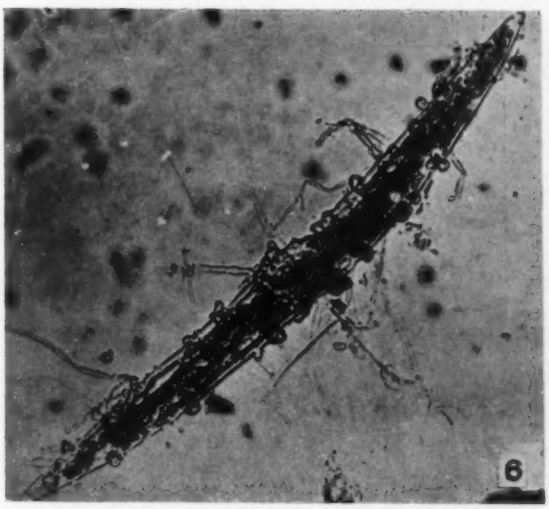
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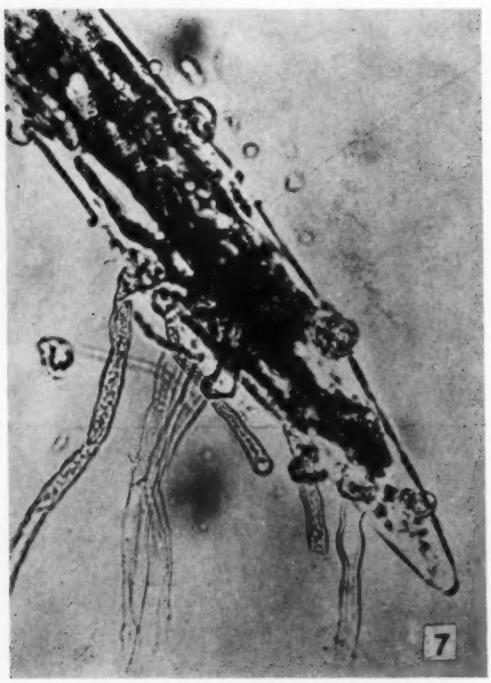
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Explanation of Plates

Plate V

Fig. 1. Closterium showing early stages of infection of *A. closterii*, ×460.

Fig. 2. Later stages of development of *A. closterii*, ×140.

Plate VI

Fig. 3. A part of the same under higher magnification showing the peculiar bulges and protuberances, ×630.

Fig. 4. Another part of the same showing ordinary external hyphæ, ×630.

Plate VII

Fig. 5. *A. closterii* showing conidia apparently borne directly from the cells without any conidiophores, ×630.

Fig. 6. Structure of the thallus of *A. closterii* seen in another closterium, ×80.

Plate VIII

Fig. 7. Part of the same under higher magnification, ×460.

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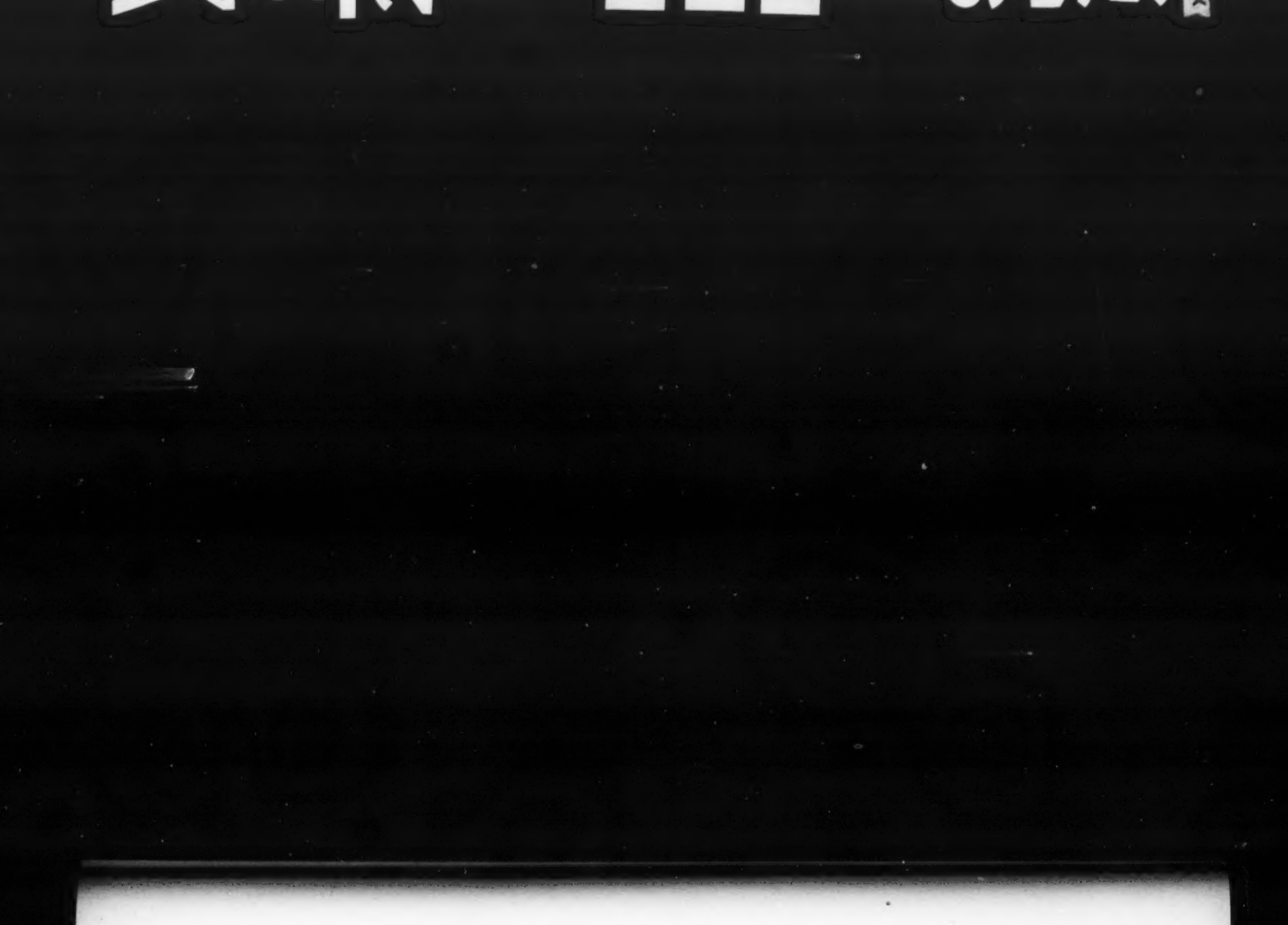

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